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THE PART PLAYED BY RECURRENT MUTATION IN EVOLUTION

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THE discovery that certain mutations occur with a measurable frequency has had a great influence on evolutionary speculation. It is the object of this paper to attempt to delimit the part which it may have played in evolution. The suggested effects have been primary and secondary. In the first place, it has been thought that as the result of recurrent mutation of a gene to its allelomorph, the new allelomorph has gradually spread through the population, displacing the original gene. In the second place, it has been pointed out that recurrent mutation of a gene would have secondary effects, even if the mutations were disadvantageous. Lethal genes, like parasites or predators, are part of the environment of the other genes. Fisher (1928) thought that the main secondary effect of disadvantageous mutations would be the accumulation of modifying genes tending to make the mutant type recessive. Some other secondary effects are suggested in this paper. Throughout I shall use the word "mutation" to denote a change in a single gene, and not with reference to such events as mutation in *Oenothera*, which generally depends on a rearrangement of the chromatin.

The evolutionary effects of mutation which will be considered are as follows:

(1) Evolution due to a mutation rate so large as to cause the spread of a disadvantageous character.

(2) Primary effects of the spread of genes nearly neutral from the point of view of natural selection: (a) Appearance of valueless but not harmful characters. (b) Disappearance of valueless but not harmful characters. (c) Disappearance of genes in the Y chromosome. (d) Disappearance of genes in the "complex" of permanently heterozygous species. (e) Disappearance of extra genes in polyploids and polysomics. (f) Primary increase of dominance.

(3) Secondary effects of frequent disadvantageous mutations: (a) Increase of dominance due to mutation of dominant allelomorphs. (b) Increase of dominance due to spread of modifying genes. (c) Selective value of polyploidy, polysomy, and duplication. (d) Male haploidy. (e) Heterogametism of male rather than female sex. (f) Concentration of mutable genes in the X chromosome. (g) Development of internal balance in the X chromosome.

In general, mutation is a necessary but not sufficient cause of evolution. Without mutation there would be no gene differences for natural selection to act upon. But the actual evolutionary trend would seem usually to be determined by selection, for the following reason.

A simple calculation shows that recurrent mutation (except of a gene so unstable as to be classifiable as multmutating) can not overcome selection of quite moderate intensity. Consider two phenotypes whose relative fitnesses are in the ratios 1 and $1 - k$, that is to say, that on the average one leaves $(1 - k)$ times as many progeny as the other. Then, if p is the probability that a gene mutates to a less fit allelomorph in the course of a life cycle, it has been shown (Haldane, 1932) that when k is small, the mutant gene will only spread through a small fraction of the population unless p is about as large as k or larger. This is true whether the gene is dominant or recessive.

Now p is usually small. The largest value found by Stadler (1929) in the case of 8 maize genes was 4×10^{-4} ,

the lowest being less than 10^{-6} . We may take 10^{-3} as an upper limit in ordinary cases. This means that a coefficient of selection k of 10^{-3} would prevent them from spreading very far. The best cases of repeated mutation of the same gene induced by abnormal environment are those of Goldschmidt (1929) and Jollos (1931). Unfortunately, the values of p can not be determined from their data, though it probably exceeded .01. But the heat used by them on *Drosophila* larvae killed many, and sterilized many more, and it seems likely that such stringent environmental conditions would wipe a species out rather than force it into a particular evolutionary path.

Very high mutation rates due to heat may perhaps have played a part in evolution in two cases. It may have caused orthogenetic evolution of species near the tropical limit of their range, and thus may possibly be partly responsible for the greater diversity of species found in tropical as compared with temperate and arctic habitats. Again, the gonads of mammals and birds are permanently at a higher temperature than is usual in other organisms. It is possible that when this temperature was first evolved, it increased the mutation rate of their genes. At the same time many new ecological niches were open, and therefore many types of mutation possessed a selective advantage. These two facts may have played a part in the very rapid evolution of mammals during the Eocene. But no definite opinion on this question is called for till a population of *Drosophila* or some other form has been shown to evolve under the influence of high temperature. If this could be experimentally shown to occur, and not to be due to selection, such an experiment would be decisive.

There are other possible causes of a very rapid mutation rate. Natural radioactivity may have caused local outbursts of mutation, but a simple calculation dispels the attractive idea that the amount of radioactive substances near the earth's surface 10^9 years ago was sufficient even to double the present mutation rates. The

evidence for mutation under chemical influences is at present inconclusive. Demereč (1929) has found that one gene may influence the mutability of another, and it is conceivable that such genes may have played a temporary part in evolution, being eliminated when a relative equilibrium was reached, and mutability ceased to be advantageous.

While, therefore, we can not deny the possibility that mutation frequency may occasionally have been large enough to counterbalance natural selection, and even to cause orthogenetic evolution of a disadvantageous character, such events must have been rare. I have pointed out elsewhere (Haldane, 1932) that many of the cases of orthogenesis ending in extinction can be explained on quite orthodox Darwinian lines. For the survival of the fittest individuals does not necessarily produce a fitter species, and competition based on such characters as embryonic or pollen tube growth rates may be expected in some cases to lead to monstrous adult forms. We must, therefore, expect that the main effects of recurrent mutation have been due to mutation rates of much less than one in a thousand per generation. It is to these that we now turn.

Where a number of allelomorphs seem to be of equal selective value, as in the case of those responsible for banding in snails and blood groups in man, their relative frequency in the population will be determined, not by the fitness of the genotypes, but by the stability of the genes. Diver (1929) has shown that the proportion of banded to unbanded *Cepaea* in England has not varied appreciably in the last few thousand years. It is therefore much less than one thousandth, and may be less than the mutation rate of one of the genes concerned. In this case, if the "banded" gene mutates less frequently than the "unbanded," it will spread until most British *Cepaea* are banded. Such cases are perhaps rare and their evolutionary importance slight, but they do seem to exist.

The main cases where mutation is a primary cause of evolution have in common the feature that mutations in one direction are weeded out by natural selection, those in the other being neutral. Consider the genetics of a useless organ such as the pelvis of a whale or the clavicle of a dog. Such organs must be practically neutral. The amount of extra calcium and phosphorus made available to a whale or dog by the complete disappearance of these bones would be extremely small, and neither whale nor dog, from the nature of their diets, can suffer from a shortage of these elements, where a small margin would be important. Of the genes affecting such vestigial organs a majority will probably affect other organs too, and be selected in accordance with these effects. A few genes will mainly affect the useless organ. Now the majority of mutations affecting an organ generally tend to bring about a reduction in its size. This is well seen in the case of the numerous genes affecting wings and bristles in *Drosophila*, and is intelligible on any biochemical theory of any gene action. The usual, though very slow, effect of mutations will thus be to diminish the size of vestiges. But as an organ becomes useless, genes which exaggerate it also become less harmful than before. For example, megalocornea would be of little harm to an animal living in complete darkness, and petalody of the stamens, though involving a slight waste of material, would not seriously handicap an apogamous plant. In spite of such occasional exceptions it is clear that the general trend will be towards the very slow progressive reduction of vestiges. As this will in some cases be due to the actual disappearance of genes, it may, in its later stages, be irreversible.

An important particular case of the disappearance by mutation of useless organs is the disappearance of the genes in the Y chromosome. There is strong reason to believe that the Y chromosome has been evolved from a chromosome with a normal complement of genes, originally homologous with the X. Such chromosomes appear

still to exist in some dioecious plants, and in fish such as *Lebistes*. On this view the Y chromosome originally differed from the X only in respect of one gene. As soon as sex differences come to depend on more than one gene, crossing over must be suppressed in the heterogametic sex, or else the Y chromosome must differ so much from the X morphologically as to render crossing over rare.

Clearly, in so far as genes in the X chromosome are fully dominant, natural selection will not oppose recessive mutations of genes in the Y chromosome. And deficiencies, that is to say, complete disappearance of genes, are stabler than ordinary recessives, which may occasionally mutate back. Thus in the long run ordinary recessive genes will be replaced by deficiencies. In some cases where dominance is incomplete the relatively small disadvantage caused by heterozygosity will not be enough to weed out recessive mutations, and these will tend to accentuate the difference between the sexes. In other cases of incomplete dominance, dominance will evolve, as pointed out later, by the Fisher effect, until the Y gene can safely be lost.

On Darlington's (1932) theory of chromosome pairing it is necessary, if the X and Y chromosomes are to pass regularly to opposite poles, that a small length of both, in the neighborhood of the spindle fiber attachment, should remain homologous, and give rise to chiasmata. In this neighborhood a few genes are to be expected in the Y chromosome, and it was here that Stern (1929a) found the normal allelomorph of "bobbed."

A somewhat analogous case occurs in permanently heterozygous plants such as *Oenothera Lamarckiana*. Here, as Darlington points out, there is a certain section in many of the ring-forming chromosomes in which crossing over rarely if ever occurs. These sections appear to carry the genes of the Renner "complex." They should show a tendency to evolve in the same way as the Y chromosome. But this will be checked by the fact that the male gametophyte, and to a less extent the female

gametophyte, is under the control of the genes carried by it, while the spermatozoon is not. This is probably one reason why the dioecious higher plants rarely show a marked dimorphism of X and Y, and these chromosomes pair regularly in prophase. Any general tendency of Y genes to mutate would be stopped by natural selection, as it would eliminate male-producing pollen. But in so far as the genes in the chromosome sections that do not cross over are indifferent to one at least of the gametophytes, there will be a tendency for a gene in one complex to disappear, while the corresponding gene in the other complex is unaltered or increases its dominance. Thus, although the degenerative process is never likely to go as far as in an animal Y chromosome, it is not surprising that many *Oenothera* species carry more lethals than are needed to produce a balanced system.

The next three processes on our list all have the same evolutionary effects, though due to rather different causes. The processes will therefore all be dealt with before the effects due to increase of dominance. When we consider any series of multiple allelomorphs we find that as regards any particular structure or function the phenotypes can be arranged in a certain order. Of course the consideration of another structure may give a different order, but there is usually a fair agreement. At one end of the series we usually find a dominant, at the other recessive forms. Thus in rodent color genetics we have the E series ranging from black through the wild gray and "Japanese" to yellow, the lightest form being recessive. On the other hand, the G (or A) series ranges from gray (in the mouse yellow) through "black and tan" to black. Here the darkest form is recessive. The wild type may be at one end of such a series or in the middle. But in the latter case we never find recessive genes on both sides. Thus in rodents we do not find two allelomorphs, one darker and one lighter than the wild form, and both recessive to it. As a general rule, however, the wild type is at one end of a series, usually the dominant end.

Goldschmidt's (1927) theory of dominance states that genes which catalyze a reaction rapidly are dominant over those which catalyze it slowly. But in many cases, e.g., "bobbed" (Stern, 1929b) a limit is reached where further speeding up of the reaction or addition to the number of genes produces no further effect on the character studied. Where this is so, and the wild type is dominant, it is clear that *minus* mutations will give rise to recessive genotypes, and, if the wild type is fitter than these, the mutant genes will be eliminated by natural selection. On the other hand, *plus* mutations will have no effect on the adult genotype (at least as regards the character considered). There will, therefore, be a tendency for them to accumulate. In the absence of the secondary effects of mutation, we should expect to find in a given locus, first recessive genes and deficiencies in small numbers, constantly being produced by mutation and abolished by selection, and second a number of allelomorphs producing almost indistinguishable phenotypes, but some possessing a great deal more activity than the minimum required to produce the wild type. The proportion of these dominant allelomorphs, producing very similar genotypes, would depend on their relative mutation rates; in other words, on their relative stabilities.

Actually, however, the process of dominance evolution is probably supported by two secondary effects. Haldane (1930) pointed out that *plus* mutations in any locus may be favored by selection provided that the original gene is not completely dominant, since the original type of gene is at a disadvantage in heterozygotes, and the new gene is not. Fisher (1931) accepts this as a supplement to the mechanism of dominance evolution which he originally suggested, namely, the spread of modifying genes which increase dominance. Wright (1929) and Haldane (1930) have criticized Fisher's original theory, and it is far from certain which of the two secondary effects is likely to have the greatest effect in increasing

dominance. It is to be noted that both the secondary causes of increased dominance are limited in their action, and cease when dominance is complete. Their intensity is proportional to the frequency of *minus* mutations in the locus concerned. If *plus* mutations are anything like equally common, the primary effect will be much more rapid than the secondary.

A particular case of the Fisher effect (secondary increase of dominance) is to be looked for in the case of sex-linked genes. The Y chromosome "empties" in the course of evolution, *i.e.*, genes borne by it disappear or at least pass into a recessive condition. This can be counteracted in two ways, by an increase of dominance of individual genes, or by the acquisition of intra-chromosomal balance. The former process would be favored if the genes in the Y chromosome disappeared one by one, the latter if they disappeared in blocks as the result of sectional deficiencies.

The evolutionary effects of increased dominance are, I think, more profound than Fisher has realized. On Goldschmidt's (1923) theory a dominant gene has a more extended sphere of action, both in space and time, than a recessive. So much of his theory may be accepted, even if we do not admit that it always contains more gene substance. Goldschmidt gives several examples from animal genetics, illustrating the wider spatial and temporal range of the more dominant gene, and they can be paralleled in plant genetics. Thus, in maize, a series of allelomorphs, not unlike the scute-achaete series in *Drosophila*, governs the distribution of anthocyanin over the cobs, silk, husks and grain, the form with the widest distribution being dominant (Anderson, 1924). In *Primula sinensis* de Winton and Haldane (1932) find that JJ flowers are fully colored, jj open white, but later assume a pale color, while Jj open white or pale, but later color up so as to overlap JJ. Thus two "doses" of J produce their effect quicker than one.

Now consider an organism in which a number of genes are just sufficient, in the homozygous condition, to produce the most advantageous adult phenotype. The result of increased dominance will be to strengthen their action so that, where possible, its range both in space and time is increased. But however much the latter is extended, there will always, on Goldschmidt's view, be a period when the homozygote is more advanced towards the adult condition than the heterozygote, even if both are quite alike in the final stage. Thus in so far as developmental abnormality is disadvantageous, the Fisher effect will always be tending to increase the activity of the genes.

This tendency may be held up on biochemical grounds, or by natural selection if such extension of activity is disadvantageous. But it will be the normal tendency. Thus, where the embryo is sheltered by an eggshell or uterus, its form has very little survival value, and the time of action of genes originally active only in the adult will tend to extend progressively back into the life cycle. Whatever be the embryonic form at any stage, there will in general be an advantage in keeping to the normal schedule of development, and thus a disadvantage in the incomplete dominance which, on Goldschmidt's theory, all heterozygotes must show in their early stages.

We thus have a sound explanation, in terms of mutation and natural selection, for the phenomena of tachygenesis and recapitulation. Genes will at first come into action rather late, but gradually extend their sphere of action in time, and on the whole the genes responsible for the phylogenetically older characters of the adult will come into action earliest. But these phenomena are of course far less likely to occur where there is an unsheltered larva in which form and function have strong survival values. We need not be surprised, for example, that limb buds appear at an earlier stage in the development of the body as a whole in Amniota than in Amphibia.

Similar arguments hold good concerning neoteny. Genes which originally determined temporary embryonic or larval characters will tend to extend their action forward into adult life. The preservation in the adult of the embryonic cranial flexure, which has been an orthogenetic trend in the evolution of the Primates, culminating in the human head, has not only been of advantage to the adults when they become climbers and later bipeds, besides permitting hypertrophy of the neopallium. It has also been a result of mutation pressure by the genes responsible for cranial flexure in the foetus; and if Fisher is right, it has been encouraged by their tendency to develop a "factor of safety" protecting them against mutant genes which would otherwise have been dangerous in the heterozygous condition.

We can thus restate, in modern terminology, Weissmann's theory of the "*Kampf der Theile*." Every gene tends to increase its activity, and the process continues until natural selection or the increased activity of other genes puts a stop to it. The process is extremely slow, simply because mutation is rather infrequent, but it will account for otherwise obscure orthogenetic tendencies.

Another tendency quite similar to that pointed out by Fisher is as follows. If a zygote contains four genes of a kind instead of two, the possibilities of harmful mutation are reduced. Thus Stadler (1929) found it much harder to produce mutations by x-rays in polyploid than diploid cereals. Duplication affecting only a few genes would confer a relatively slight advantage. But duplication of a large section, polysomy of a whole chromosome, or polyploidy, might confer a considerable advantage, provided it caused neither unbalance nor sterility. Whether this advantage is sufficient to be of evolutionary importance is not clear, but the possibility exists. In any case such an evolutionary step, on whatever grounds the new type may survive, leaves such of the doubled genes as are fully dominant at the mercy of mutation, provided meiosis is so regular that each chromosomal locus finds a definite mate. For recessive genes will only be elimi-

nated if recessive mutations occur both in the original locus and the duplication, and all four of the recessive genes occur in one zygote. While the duplication or polyploidy may be preserved because it protects against mutations in some thousands of loci at once, the single genes will tend to disappear one at a time because they only protect one locus apiece. The final result will be that most of the genes will return to the diploid condition, but their spatial relations will have been greatly altered. The process is therefore probably cyclical.

We now come to a group of secondary effects connected with the cytology of sex. Consider an organism such as the bee, in which the male is haploid. Recessive lethal and semi-lethal gene mutations presumably occur, and kill off a certain number of males. But they can never kill a female (or diploid neuter) unless they are sex-limited in their incidence. As there appears to be an excess of drones over the minimum number needed, such lethal mutations, provided they are fully recessive, are of no disadvantage, and indeed of some slight advantage, to the species. If, on the other hand, the male were diploid and the female haploid, lethals would diminish the number of females, and thus be disadvantageous. There may well be sound physiological reasons why haploid animals are never female, but the above argument furnishes an additional reason for this fact.

The same argument applies, with less force, to sex-linked genes in general. When once the majority of the genes in the Y chromosome have become inactive, recessive lethal mutations in the X chromosome will only kill off males. So in any species where more males exist than are needed to secure the fertilization of all females, it is advantageous that the male should be the heterogametic sex. We should therefore expect to find female heterogametism mainly developed in groups where monogamy is the rule. In such a case the killing off of males would have as serious an effect on fertility as the killing off of females. Actually monogamy is the usual condition in birds, and in *Lepidoptera* it is rather uncommon for one

male to fertilize several females. These are, of course, the two principal groups in which the female is heterogametic.

In a species where the male is heterogametic and males are produced in excess, there will clearly be a small advantage in any rearrangement of the genes by which those that frequently give rise to lethal mutations come to lie in the X chromosome. Such unstable genes will then only kill males. Owing to the greater ease of detecting sex-linked lethals, as compared with autosomal lethals, the evidence from *Drosophila* which at first sight suggests the truth of this deduction is of course worthless. But the human evidence points in this direction. Sex-linked recessives, such as haemophilia and Leber's disease, which would be semi-lethal in a state of nature, are fairly common. If loci giving lethal mutants were equally common in all chromosomes such defects should only be about one twenty-third as frequent as autosomal recessives recognizable by their causing congenital abnormalities in several members of a family. This is almost certainly not the case. And the fact is not surprising. In a stage of social evolution where a number of young males can get no mates and may be a nuisance to the herd, a few sex-linked lethals may be a positive advantage to the species, as is the presence of genes conducting to cancer, which kills off superfluous old men and women, at the present day.

A probably still more important influence on gene arrangement is the tendency discovered by Muller (1930) for internal balance to develop in the X chromosome, thus minimizing the differences between XX and XY individuals. This tendency must be at work in all zygotes of at least one sex, while the former is only operative in those carrying a lethal or semi-lethal gene. The tendency to balance may, however, be regarded as a secondary effect of the accumulation of recessive lethals in the Y chromosome. Indeed this accumulation can only proceed as the X develops internal balance.

It is noteworthy that the zygote of higher plants is to a large extent automatically protected against lethals by the fact that many genes are active in the male gametophyte. The majority of the zygotic lethals known in plants kill by suppressing the formation of chlorophyll, which is of course irrelevant to the gametophyte. Those which interfere with other fundamental cell functions will usually kill pollen tubes, and never attain homozygosity in a zygote. Moreover, they will rapidly be eliminated, even in the heterozygous condition, the number being halved in each generation. In so far as this is true we may expect that recurrent lethal mutation has been less important as a factor in the evolution of higher plants than of animals. Certainly there is rather little evidence for tachygenesis in plant evolution or recapitulation in plant development.

It must at once be admitted that much of this paper is frankly speculative, and that some of the speculations may prove groundless. As Wright (1929) has pointed out, the effects of recurrent mutation are at best extremely slow. Many of them may prove as unimportant in organic evolution as the tides raised by the planets on the sun have been in cosmic evolution. Others may turn out to have had as marked effects as have the tides in the evolution of the earth-moon system. Only careful and quantitative work will decide. Such effects, if they exist on a sufficient scale, will explain certain orthogenetic phenomena which have seemed to demand a vital urge, racial memory, and so on. But just because the theories here put forward are in some degree intellectually satisfying, it is important that they should not be accepted without stringent examination.

SUMMARY

Recurrent mutations not only provide the material for selection to act upon. They may give rise to primary and secondary effects, the former due to the accumulation of mutant genes, the latter to the selective value of conditions which protect the organism against lethal genes.

Among the phenomena which can be accounted for by these phenomena are the disappearance of useless organs, recapitulation and the fact that the heterogametic sex is usually male.

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CORTICAL CYTOPLASM AND EVOLUTION

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I

THE fact of evolution is as firmly established as any in biology. Evidence for its support is available from many lines of biological inquiry. However, it is frequently maintained that the case in favor of evolution is too greatly built upon evidence from the past. The evidence from paleontology has therefore been objected to here in America, especially by the anti-evolutionists. It is a curious fact, though not difficult of explanation, that paleontology, together with astronomy, intrigues the American public: it has more interest in these two branches of science than one would at first thought suspect. It thus comes about that a great deal of work is done in paleontology which allures a public often capable of adducing adverse criticism meriting consideration. We must also admit, I think, that, due to the extravagant and sometimes erroneous claims of certain American paleontologists, the anti-evolution forces have justification for the objections which they raise to the evidence of the fact of evolution gathered from work in paleontology. But surely one does not need point out that the evidence in support of evolution is not wholly derived from paleontology; surely it would be a mistake not to appraise justly the evidence from other branches of biology. Not only distribution in time but also distribution in space and also taxonomy, comparative anatomy, embryology, physiology and biochemistry yield each its mead of evidence in support of the proposition that the world of living things, as we know it to-day, is the result of an evolutionary process rather than of special creation. Moreover, the contributions of modern genetics yield evidence of the fact of evolution. In front of all, genetics is experimental evolution.

Among biologists exists the almost unanimous verdict that evolution took place. When, however, we turn to the question concerning the cause of evolution we find no prevailing opinion. Thus, it would be difficult to assemble any substantial number of biologists to-day who could agree as to the manner in which evolution came about. It may be that in time genetics will furnish us with the clue which will serve to solve this problem—for the evidence from this branch of biology, though in a certain sense small, is after all, when we consider its youth, not only large but significant. This one must hope because tangible work of to-day is more satisfactory than speculation concerning the past. And yet, such speculation will always be alluring. The drama of the universe in the act now before us is a tremendously moving spectacle, but the prologue to its pageantry is also capable of moving the dullest imagination. One need, therefore, make no apology, despite the clamor of existing theories, in voicing a note concerning the origin of the world of living things.

II

Even if we were to-day suddenly to agree upon one of the existing theories of the cause of evolution, or should make a theory which would encompass some of the existing ones, or if, finally, genetics were to give us the answer, there would still remain questions to be answered. These at present can not be answered, and some of them will never be answered until out of non-living substance a living thing is made. One fundamental question therefore still remains: namely, how out of non-living matter did life arise?

The combination of chemical compounds from the environment to make up the first living thing must obviously have meant a separation from the environment, that is, the combination must have been peculiar, both physically and chemically; otherwise, there never could have come about its separation and the maintenance of

its integrity apart from the environment. Now the moment that this peculiar combination of compounds arose, there must have begun reactions or responses of it to the environment—especially to temperature, to gases and to electrolytes. The chief characteristic of this original substance was its peculiar and complex organization, which set it apart from its environment, but at the same time it must have been responsive to environmental changes. Indeed, these environmental changes must in the first instance have brought about the combination of compounds peculiar to living substance, and in the second place must have conditioned its activity.

If we assume that this original mass of primitive protoplasm at first showed no high degree of differentiation, we can scarcely imagine it as a homogeneous structure throughout. As such it could not endure for any great length of time. As a matter of fact, the moment that we assume that a combination of chemical compounds was separated out from the environment as a living system we must, it seems to me, postulate some differentiation in the mass—which differentiation would serve to keep the organism or combination of compounds intact.

If, however, we could imagine such a combination of compounds separated from the environment as purely homogeneous throughout, then there must soon have come a time when factors in the environment played upon this structure and so modified, if they did not determine, its behavior. Certainly, it would be difficult to imagine even a non-living structure made up of the same elements or compounds found in the environment and maintaining its separateness from that of the environment without some structural difference from the environment. Any hypothetical drop of living substance, therefore, that we can imagine suspended in water or other solution must in some way have an organization which keeps it apart from its environment. The moment that this combination of compounds assumed life, it had response to that environ-

ment, both because it arose from the environment and must live in it.¹

Environment and organism are one; neither can be separated from the other. Living substance can not be considered abstracted either from time or from space. Two predominating characteristics exhibited by living organisms are: first, those changes which are time-ordered; and second, those which are environment-conditioned. Thus, the organism—a single cell, for example—changes from moment to moment and the rate of such changes becomes its differentiating characteristic; and though these changes tend to run in one direction—building up protoplasm from simpler compounds while life lasts—the environment plays a part in yielding up the raw material for these changes and setting the conditions which make possible the reactions in the living substance. In a certain sense we should not speak of the fitness of the environment or the fitness of the organism: rather, we should regard organism and environment as one reacting system. However, in a measure at least, the reactions in this system tend to run in the direction of the living component.

The play of factors in the environment—temperature, gases and electrolytes—upon the living organism must be first on the cytoplasmic surface. Even if we assume that the primordial living thing was a mass of homogeneous protoplasm structurally the same throughout, there must have early arisen a differentiation between surface and interior—a spatial differentiation. Moreover, the constant interchange between environment and organism must mean reactions which take place first in the more superficially located cytoplasmic structure; these reactions would condition succeeding ones in the

¹ Although we must admit the possibility of life below size capable of observation under the highest powers of the microscope, we must also admit that we know little concerning the organization or structure of such ultra-microscopic organisms. If this be true, then we might most profitably begin with a hypothetical organism whose size is within the range of resolution by the microscope. Nevertheless, what is said of such a hypothetical structure might also be true of an ultra-microscopic one.

endoplasm. Hence the differentiation was not merely spatial. The first step in the evolutionary process, then, was a differentiation of the cytoplasm into ectoplasm and endoplasm.

III

The question concerning the origin of living substance out of non-living is in turn related to others:

Was there a single parent stem of living substance which gave rise to both animals and plants, or did animals and plants arise independently of each other? If they did arise independently, was there one parent stem for plants and one for animals, or were there several stems of origin for each kingdom? Since answers to these questions to-day are purely speculative, one speculation is as good as another; I therefore here postulate that animals and plants arose from one primordial living thing.

There are relatively few fundamental functions of the living protoplast. Among these are contraction, conduction, respiration and nutrition. The primordial contraction, or let us say that exhibited by the egg or the protozoan cell, involves the cell interior to a secondary degree only. For example, the unfertilized egg of *Arbacia* is highly elastic. It can be drawn out into a fine strand; it returns to its original shape without loss of fertilization capacity when the strain is removed. This contractility, however, is lost after insemination with those changes at the surface incident to fertilization. The contractile power of an amoeba is due to the ectoplasm. The cilia of ciliated protozoa are ectoplasmic structures.

Closely bound up with contraction is the phenomenon of conduction. Here again we find an ectoplasmic function. The transferal of the effect of a stimulus is largely, if not wholly, ectoplasmic. In the fertilization of the egg it can be shown that the effect of great rapidity is confined to the surface. There is evidence that an egg without ectoplasm is incapable of fertilization. Therefore, the initial effect of insemination travels only over the surface because it can not involve the interior.

Once fertilization is effected the egg can not be fertilized again because the local fertilization-reaction propagates a wave which runs over the surface and so bars reaction with additional spermatozoa. This is evidence for the differentiation between the ectoplasm and the endoplasm. In tissues highly endowed with conductivity, as well as in tissues highly endowed with contractility, the predominating characteristic is relatively large surface area. By and large, it is reasonable to assume that both contractility and conductivity are greater in surface-rich than in surface-poor protoplasm.

If we turn to those animals which possess nervous tissue, we note that this tissue—and also where present the nervous system—arises from the ectoderm cells. In many animals which possess a nervous system the eggs show unequal cleavage both as to size of the blastomeres and as to rate of cleavage. In all these eggs the smaller blastomeres with the greater rate of cleavage are ectoderm cells. Now it would seem to be good reasoning to take the position that rapidly dividing cells are such because they lack or have less inert metaplastic substances. I do not mean by this merely the bulk of yolk, for example, but rather the distribution and the kind of yolk (and other metaplastic substances) in a given cell; that is, the presence of yolk *per se* is not necessarily a hindrance to cleavage. Instead, it is the distribution of this yolk and its physical make-up which interfere with the cleavage process. On the whole, therefore, we may conclude that the most rapidly reacting tissue among metazoa is the nervous tissue, and that this arises from the ectodermal cells, which in turn are the most rapidly developing cells of the zygote. There is some evidence which indicates that those cells among all cells of the zygote divide most rapidly which possess most hyaline cytoplasm. In other words, the ectoplasm, which is the surface-located hyaline substance of the egg, has a great deal to do with determining the rate of cleavage and also the direction of the cleavage furrows.

The subsequent differentiation from a hypothetical first form of life into animal or plant we may suppose came about through the greater exaggeration of contraction and of conduction by that form which evolved as animal. A deviation or less emphasis on these brought plants as such into being. It is surely on the side of the nervous tissue system that a great difference lies between animals and plants.

With respect to cellular oxidations, we have the very beautiful work by Warburg which establishes that cellular oxidation is a function of cytoplasmic structure. Again it is reasonable to assume that oxygen coming into the cell makes first some union with the superficial cytoplasm. Certainly this assumption is more reasonable than that which states that oxygen enters and remains inert until it reaches the nucleus, formerly considered the seat of intra-cellular oxidations.

Animal evolution advanced rapidly or slowly, to a higher or lower stage, depending upon the degree of ectoplasmic behavior exhibited as contraction and conduction. Animals to-day differ largely because of differences in these two manifestations of life. Plants to-day differ from animals largely on the side of these two manifestations, since they possess them to a less degree. Animals exaggerate the fundamental phenomena of contraction and conduction, hence the origin and evolution of muscle and nerve tissue; plants do not.

Now of three fundamental life-processes—contraction, conduction and respiration—respiration may be regarded as primary: on it depends all vital activity. Respiration, the same in both animals and plants, may yet have been responsible for the early splitting off of the plant and animal kingdoms from the first hypothetical living thing. That is, those individuals of the first form of life possessing most rapid rates of oxygen consumption tended to oxidize themselves, or as cannibals, their like. Some, because of lesser intake of oxygen, tended to accumulate CO₂, and thus developed photosynthetic power as a means of protection. With this went also the building up from

CO₂ and water of carbohydrate polymers to form complex compounds, including cellulose. The presence of cellulose determined the organization of the cytoplasm found in higher plants—that is, a cytoplasm of peripheral location enclosing a vacuome.

Speculation concerning the origin of the world of animals and plants as we know it to-day must remain as speculation until we can make a living thing. In a sense, therefore, such speculation is fruitless. We tend always to explain the past history of animal life in terms of our knowledge of life to-day. If it be true that the primitive cells of to-day, protozoa and egg shells, possess an organization into ecto- and endo-plasm which shows a physiological differentiation, thus placing the scene of vital phenomena in the cortex or ectoplasm, then the explanation here attempted merits some consideration.²

IV

Conceptions of protoplasm as a rigidly mechanical system have dimmed our vision. We have often striven to prove life as wholly mechanistic, starting with the hypothesis that organisms are machines! Thus we overlook the organo-dynamics of protoplasm—its power to organize itself. Living substance is such because it possesses this organization—something more than the sum of all its minutest parts. Our refined and particularistic physico-chemical studies, beautiful though they are, for the most part fail because they do not encompass that residuum left after electrons and atoms and molecules and compounds even have been studied as such. It is this residuum, the organization of protoplasm, which is its predominant characteristic and which places biology in a category quite apart from physics and chemistry.

² The reader is referred to my papers—"Die Rolle des kortikalen Cytoplasmas bei vitalen Erscheinungen" (*Naturwissenschaften*, 1931, H. 48, 49 and 50) for a discussion of the rôle of the cortex in these phenomena. My paper, "On the Origin of Mutations" (*Amer. Nat.*, Vol. 66, 1932) is a discussion of the effect of external agents on cytoplasm as the cause of the altered chromosome behavior in mutations: every mutation due to chromosomal behavior is preceded by cortical modification. From this point of view, "enduring modifications" and mutations differ in degree only since the cause of both lies in the cytoplasm.

To be sure, protoplasm is a physico-chemical system. As such it must finally yield to analysis by physicists and chemists. Nevertheless, it is, in my judgment, a mistake to predicate explanations of vital phenomena in terms of present-day conceptions prevailing in physics. Even if there were no disagreement among physicists concerning the structure of the atom and the behavior of electrons, it would still be hazardous for biologists to offer explanations of protoplasmic activity in terms of physical theory. Therefore, I do not believe that I deal in "hyperphysics" when I say that up to now purely physico-chemical analysis of protoplasmic behavior by biologists has not given results sufficient to indicate an answer to the fundamental problem in biology; nor is it barren vitalism to say that there is something remaining in the behavior of protoplasm which our physico-chemical studies leave unexplained. This "something" is the peculiar organization of protoplasm. The successful answer to the questions concerning the origin and cause of vital phenomena thus waits upon our sharper definition of protoplasmic structure.

Now the structure of cytoplasm varies, and this variation frequently depends upon the worker who describes the structure: if he work on living cells, he gives us one picture which may be true for the particular cell which attracts his attention, but not true for all cells; if he work with fixed cells, his picture may be true or false, depending upon the excellence or poverty of his technique. Frequently, workers do not distinguish between the metaplastic substances and the ground substance, or true cytoplasm. Thus, on the basis of observations made on improperly fixed material, workers tell us that protoplasm is made up of holes; whereas what is true is that the action of the fixative in such cases has dissolved out the yolk and the oil, leaving strands or granules among the empty spaces. In this discussion I define the cytoplasm as all the material of the cell outside of the nucleus minus the cytoplasmic inclusions—oil-drops, yolk, food granules, mitochondria, Golgi bodies and the like.

The simplest types of cells are undoubtedly free-living cells—as, for example, the protozoa and egg cells. Certainly in these latter the ground substance, as revealed by the study of them after centrifuging, is a clear homogeneous matrix in zones of which are suspended the various cytoplasmic inclusions. That this ground substance is the cytoplasm *par excellence*, we know from the fact that hyaline pieces of it, from certain eggs, entirely devoid of cytoplasmic inclusions, are capable of development which except for the size of the larvae is scarcely distinguishable from that of the whole egg.

In all these free-living cells there is a cytoplasmic differentiation: that is, the cytoplasm is made up of ectoplasm and endoplasm. This differentiation is a structural one. It is, moreover, physiological. Indeed, in some cells which do not clearly exhibit at a given moment in their life history a structural ecto- endo-plasmic differentiation, there reveals itself a behavior which marks a difference between ectoplasm and endoplasm. Finally, this differentiation varies from moment to moment during the life history of all cells. We must therefore bear in mind that, in front of all, the living substance is a time-thing.³

It is a fair assumption that protozoa are the most primitive animals existing to-day. To this most of us are agreed. Moreover, the egg cell is the adult metazoon in its simplest and most primitive form. Since these primitive cells exhibit so clearly ecto- endo-plasmic differentiation, it may be that this possesses some significance for a theory concerning the origin and evolution of living things. I therefore suggest that the first living thing arose out of the environment through a differentiation between the surface and the interior of the complex of compounds of which it was made; that animals and plants diverged from this primordial form through differences in structure (and behavior) of the cortical cytoplasm; in short, that cortical cytoplasm was and still is a factor in evolution.

³ See also Bayliss, *Principles of General Physiology*, Longmans Green, 1915.

A POSSIBLE RELATION BETWEEN THE PRODUCTION OF ANIMAL PLANKTON AND THE CURRENT-SYSTEM OF THE SEA¹

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DAMAS'S CIRCULATION HYPOTHESIS

I

FROM a number of investigations carried out during the last 50 years we know that every planktonic species, animal or plant, has its own characteristic geographical distribution. Only very few of the papers published on this subject attempt, however, to discuss the reason for this phenomenon. A very interesting exception in this respect forms a paper by Damas (1905), where Damas not only raises the problem of the specific distribution of plankton organisms, but also suggests a method for its possible solution. Damas' questions we may form in the following way: Why are the species of plankton animals in their distribution limited to distinct areas of the sea? Why is it that these animals, which we know may be carried far away with the currents, are not within a short time evenly distributed from pole to pole?

Damas's investigations included three important species from the southern Norwegian Sea, *Calanus finmarchicus* (Gunner.), *Calanus hyperboreus* Krøyer and *Pseudocalanus elongatus* Boeck. His treatment of the *Calanus finmarchicus* most clearly demonstrates his method. For this species Damas succeeded in determining 11 moulting stages (later investigations have proved the existence of 12 stages). The occurrence of eggs, as well as each of the moulting stages or groups of such, and also the adult animals were now by Damas mapped out separately. These charts Damas now compared with

¹ Read before the Academy of Science, Oslo, October 3, 1930.

charts showing the differences in external conditions of the area investigated, hoping by this method to find an interrelationship between distribution and environment.

Damas's charts of the distribution of eggs, males and females of the species mentioned (Fig. 6) proved the existence of a large and distinct spawning area during May-June, 1904, a spawning area which was found to be situated within the surface isothermes for 2° and 6° C. The main spawning was even found to occur between the narrow limits of 4° and 6°. The larval stages from this spawning area had, on the other hand, a far wider distribution than the eggs. They followed the current northwards and eastwards through the Norwegian Sea.

In order to give a feasible explanation of the phenomenon just described, Damas put forward his circulation hypothesis, a hypothesis which he also tried to extend to other species and other areas. The foundation for this hypothesis was the discovery by Helland-Hansen and Nansen of the great cyclonic current system of the waters of the southern part of the Norwegian Sea, which, however, in 1905 had not yet been published. Damas assumed that the whole life cycle of the species in this area depended upon this cyclonic system. Spawning could apparently only take place within the limits of temperature which he had found inside the spawning area. The new generation derived from the spawning was first carried with the current away from the spawning area, and later with the cyclonic current back again to the same area and to the same physical conditions where spawning could once more take place and another generation could then start its drift. The necessary time for each drift along the course of the cyclonic system and each generation would, according to Damas, have to be one year.

II

From a biological and hydrographical point of view grave doubts may be raised against Damas's famous

circulation hypothesis. Our present knowledge of the temperatures at which the spawning of *Calanus finmarchicus* takes place thus forms a conclusive objection. If we compare the different temperatures of the areas from which this wide-spread species has regularly been recorded, a considerable variation in spawning temperatures is found. The papers by Farran (1927) and Russel (1928) clearly show that the spawning at the English coast takes place at a temperature of about 10° or more. The frequent occurrence of the species during the whole year in more southern waters proves that the upper limits of spawning are not herewith reached. In the Lofoten area, at the Norwegian coast, from where I shall below give some results from my own investigations, I have actually found spawning females and eggs of the earliest cleavage stages in waters of a temperature down to 1°.22. The frequent occurrence of the species in waters where even lower temperatures are predominating suggest still lower limits. Certainly, it is thus very improbable that in the southern Norwegian Sea—among holoplanktonic species—we should find a local race pre-adapted to spawn in the narrow temperature interval of 4°–6°.

SPAWNING OF *Calanus finmarchicus* AND *Calanus hyperboreus* IN THE LOFOTEN AREA

An extensive material collected by the Norwegian Fishery Board in the Lofoten area, together with materials collected by Nordgård from the same area in 1899 and 1900, has given me an opportunity for investigating the spawning areas of the two species, *Calanus finmarchicus* and *Calanus hyperboreus*, according to the methods of Damas.

Damas gives in his paper only very few details as to his methods for determining the younger stages. The first task of my work has therefore been to give a more complete description of the younger stages and to work out a method for the determination of the quantities of

each stage present in the sample. A morphological difference was in the two closely related species only found in the structure of the eggs, while in the early larval stages (six nauplius and three first copepodid stages) no such difference could be found. By measuring the carapace, however, a decided difference in size was found, and by this means a separation of the species and a quantitative determination can be effected.

By using this method I have succeeded in mapping out the spawning areas for the two species during their spawning time, March to April (Fig. 1).

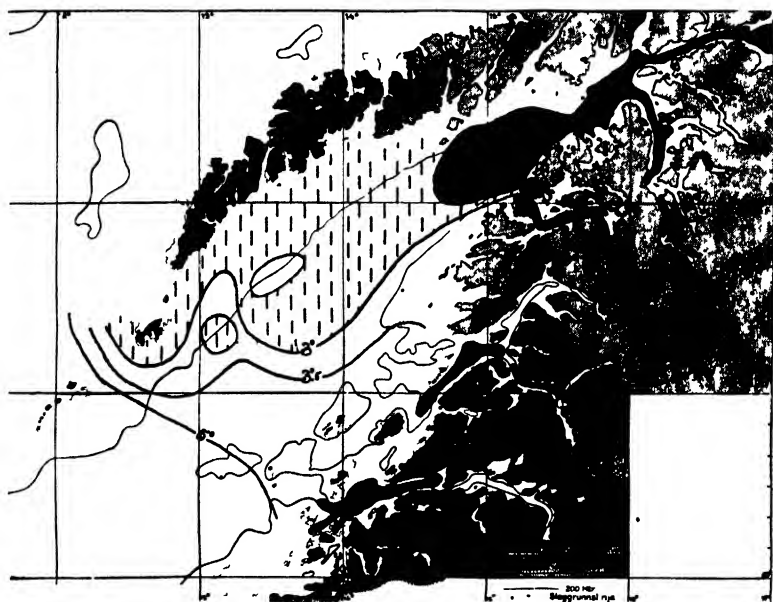


FIG. 1. The area investigated with the surface isotherms for March to April, 1922. The darkened areas show the centers where rich hauls have been made during winter time for both species investigated, and to which the spawning of *Calanus hyperboreus* is limited. The area shaded is the cold area into which most numbers of *Calanus hyperboreus*, females and larval stages, are carried after spawning time, and where the main spawning of *Calanus finmarchicus* takes place.

Spawning *Calanus finmarchicus* and their eggs were found in every locality investigated, but the numbers of females and eggs show a great deal of variation within

different parts of the area. A cross-section on the 9th of April, 1922 (Fig. 2), where the numbers of females and eggs of this species have been recorded graphically with the temperatures of the water, show a marked in-

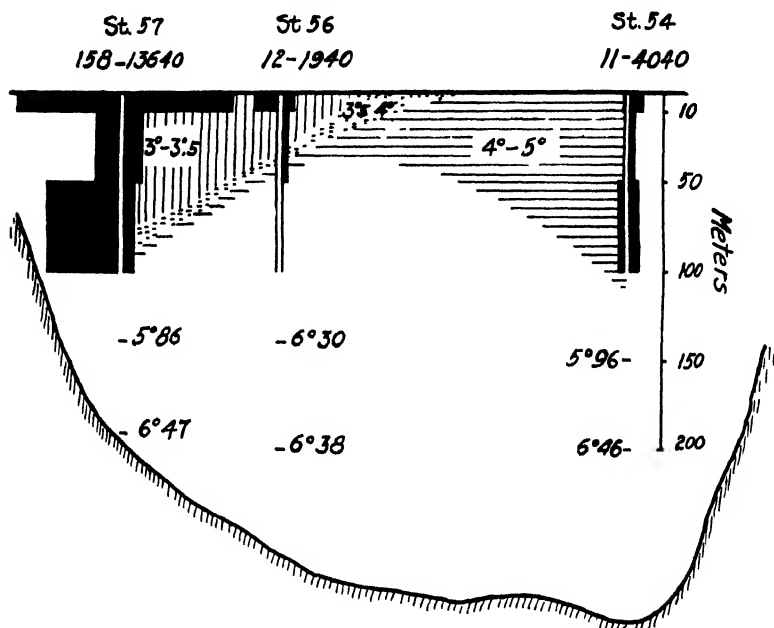


FIG. 2. Cross-section from the outer part of the Lofoten area on the 9th of April, 1922, with temperatures, together with the number of females (to the left) and eggs (to the right) of *Calanus finmarchicus* calculated as average numbers in haul of 100 meters. Total numbers are given beneath the number of station.

TABLE 1

AVERAGE NUMBERS OF EGGS AND FEMALES OF *Calanus finmarchicus*, MARCH TO APRIL, 1922, PLOTTED AGAINST SURFACE TEMPERATURES OF DIFFERENT PARTS OF THE AREA INVESTIGATED. N = NUMBER OF HAULS.

Surface temperature	Vertical hauls Nansen closing net			Surface hauls 5 minutes	
	n =	Eggs	♀ ♀	n =	♀ ♀
1°-5-3°-5	39	5972	98	20	8400
3°-5-5°	19	490	2.8		.
More than 5°	9	560	5.9		.
More than 3°-5			.	28	98

crease in the numbers in the northwestern part of the section, where the surface temperature is less than 3° . This characteristic feature has been found in all cases investigated. Table 1 thus gives the average numbers of eggs and females in March–April, 1922, plotted against intervals of surface temperatures. From waters of a surface temperature below $3^{\circ}.5$ the number of individuals are strikingly predominant. Apparently we have here a characteristic spawning area very much like the spawning area described by Damas. The proportion of eggs and females show, however, no marked difference in parts of the entire area investigated. The predominant spawning in the northern area of cold waters is thus simply due to the great excess in numbers of spawning females found there; the fewer specimens spawning freely outside this part of the area clearly shows that no limiting factor exists for the spawning process itself.

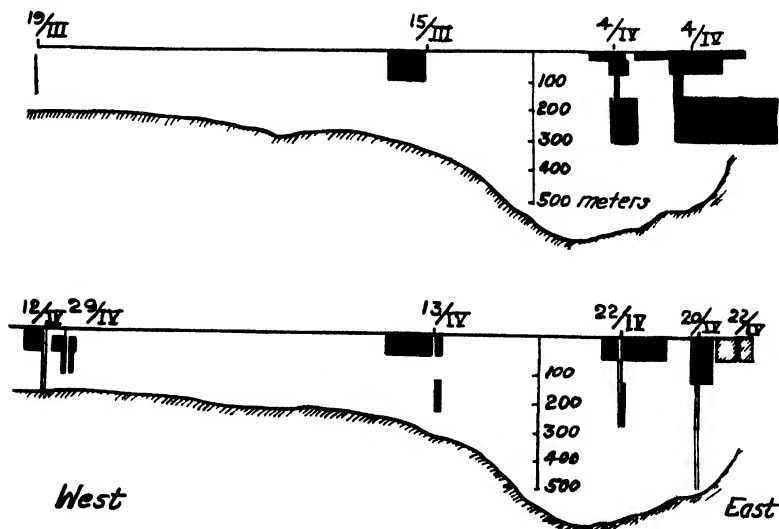


FIG. 3. Two sections along the Lofoten area, March to April, 1929, with the numbers of old generation of *Calanus hyperboreus* (to the right) and *Calanus finmarchicus* (to the left) calculated as average numbers in hauls of 100 meters. The first section was investigated at the time when *Calanus finmarchicus* had finished the vertical migration. *Calanus hyperboreus* in case of vertical migration. The second section shows how the numbers decrease inside the winter area and increase outside after the vertical migration has been finished.

For *Calanus hyperboreus* the spawning area was found to be strongly limited to the inner parts of the area, mostly inside the surface isotherm of 2° (Figs. 1 and 3a). In this species the many samples examined from the years 1922, 1923, 1924 and 1929 clearly reveal that no spawning takes place outside this part of the area; the species in question is here not found outside the inner area before the first days of April, and the samples are then found to consist of females which already have spawned (recognized by their wide oviducts) and later larval stages.

An interesting fact in the biology of this species is a scarce stock of actually spawning females with their newly spawned eggs, which was found inside the spawning area in hauls from below 100 or 200 meters more than a month after the spawning in the upper layers had been finished. The temperature during this spawning was from $6^{\circ}.5-7^{\circ}$. In cultures I have, moreover, succeeded in observing the mature females spawning in temperatures from $-1^{\circ}.5-7^{\circ}.5$. If the temperature represented the limiting factor for the spawning of this species, spawning would, according to these observations, take place not only in the whole area investigated by me, but also in most parts of the Norwegian Sea and in wide areas in the

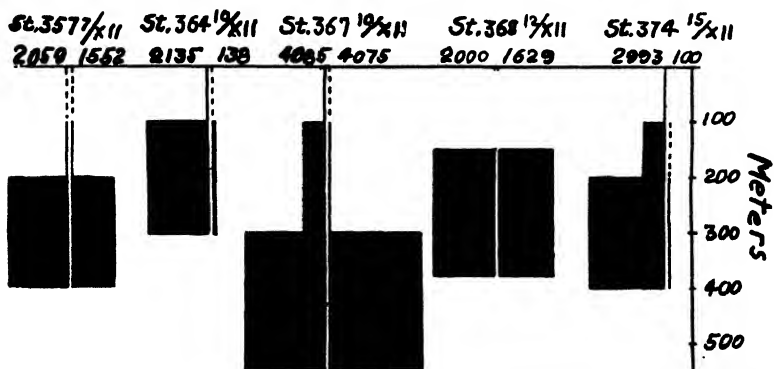


FIG. 4. Vertical distribution of *Calanus finmarchicus* (to the left) and *Calanus hyperboreus* (to the right), December, 1928. The three stations 364, 367 and 368 are from the Lofoten area ($67^{\circ}-68^{\circ}.30'N$), station 357 from a fjord further north ($69^{\circ}N$) and station 374 from Møre ($63^{\circ}N$).

north Atlantic. About the same conclusions would be reached regarding the salinities at which spawning has been observed.

What, then, is the reason for the peculiar arrangement of the two species during the spawning time?

A section from December, 1928 (Fig. 4), shows the large numbers which at this time of the year are accommodated in great depths. *Calanus hyperboreus* is only found in hauls which are representing waters deeper than 300 meters; *Calanus finmarchicus* is most frequent in the deepest hauls, but is also met with in considerable numbers in waters up to 200 meters. This type of vertical distribution, which has always been found in materials from December, January and the two first weeks of February, lead to an interesting conclusion. The absence of *Calanus hyperboreus* in the outer parts of Norwegian coast waters before the 15th of March (found by Nordgård (1899), Ruud (1929), from my materials and others) are quite simply explained by the fact that those great depths, which are a matter of necessity to the species at this time of the year, do not exist in these parts of the ocean. In *Calanus finmarchicus* the materials give the same picture, only not so marked as in the preceding species. A few specimens have during the winter been caught outside the deep areas by Nordgård, Ruud and by myself, but the numbers caught are always less than 1 per cent. of the numbers from the rich hauls in the deeper water masses.

This winter situation is in both species interrupted by an active vertical migration of the animals in spring.

Fig. 5 shows the type of vertical distribution in *Calanus finmarchicus* before and after the migration. The intermediate stages—catches from the period of actual migration—are fairly well demonstrated in Nordgård's materials and partly in my own materials (e.g., *Calanus hyperboreus* in Fig. 3a). In this way it has been possible to determine the time at which the migrations take place. In the case of *Calanus finmarchicus* migration is

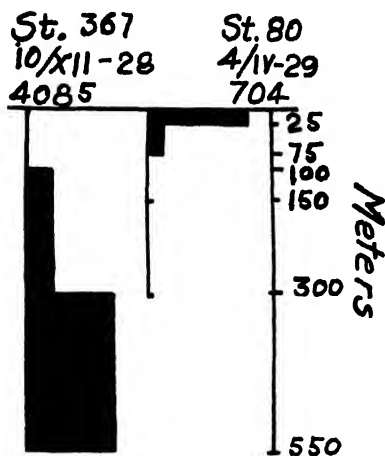


FIG. 5. Different type of vertical distribution in *Calanus finmarchicus* in the Lofoten area in December and April. The total numbers caught are in each case found beneath the number of the station.

finished before the 15th of March, in *Calanus hyperboreus* not before the 15th of April, the difference in time between the two species thus being about one month.

In the deep water where the water masses are but comparatively slowly renewed, the animals are well protected against the stronger effects of current. As soon, however, as they by vertical migration reach the surface layers, they arrive in water masses which, on account of the flow of fresh water, are forced away from the coast. The total numbers of individuals caught in the hauls from bottom to surface at a station thus always show a marked decrease during and after the migration in those places where great numbers were found to be stationary during the winter. In the southern part of the Lofoten area, where the coastal current, carrying water of comparatively high temperature and high salinities, runs into the area, nearly all specimens from the winter time are washed away shortly after the migration. At one station here (Økssund) I obtained on the 10th of December, 1928, 4,085 specimens of *Calanus finmarchicus* before the migration. Nordgård obtained 1,443 on the 17th of February, 1899, during the period of migration and only

186 on the 29th of March, 1899, after the migration. In April, 1922, several catches from this same locality have shown me none or only single specimens. In the fjord of the northeastern part of the area, where the currents are of more local origin, more specimens are left after the migration (Fig. 3). On an average I have found that about 19/20 of the winter stock are carried away, only 1/20 are left behind in the original area.

Simultaneously with the decrease in numbers inside the areas where the species are found during winter, an increase has been found in certain areas outside, especially in the northern part, caused by the transport with the currents from the inner parts.

The average numbers found in different months of winter and spring thus gives us the means of defining the areas from which the species disappear after the migration and to which areas they are carried away.

Another factor which is of great interest for the definition of the extent of the spawning areas has been found in the study of the breeding season of the species. The females of *Calanus hyperboreus* have their oviducts filled with eggs, and the copulation takes place in January and the first week of February. During the three last weeks of February they are only found with unripe eggs in the later stages of growth. The males die off and disappear from the plankton before the vertical migration. During migration the composition of the stock was in 1929 found to consist of 57 per cent. females and 43 per cent. belonging to the two last larval stages. The spawning takes place during or immediately after the vertical migration, and males, or females with eggs, have—in spite of the excess of larval stages—never been found after the migration.

Thus in *Calanus hyperboreus* but one factor appears to determine the extension of the spawning area, namely, the distribution of the species during winter time.

In *Calanus finmarchicus* this is different. On an average, this species—as already mentioned—undertakes the

vertical migration a month earlier than the preceding species, while the appearance of eggs, in the oviducts of the females and in the samples collected, shows that it spawns more than a month later. Two factors are accordingly found determining the spawning area of this species, namely, the winter area of distribution and more than two months' exposure to the scattering effect of the surface currents.

The phenomenon that the spawning areas are found limited to certain isotherms and isohalines thus appears to obtain a quite natural explanation. The limiting isotherms and isohalines are merely these characteristic limits of different currents, well known to oceanographers, in this case the limits of cold and fresh water carrying the species away from the inner coastal areas.

The chief features of the seasonal distribution and the production of individuals of the two species investigated are, as will be seen, due to a combined biological-hydrographical mechanism, the details of which, as hitherto observed, are now being published in "Report on the Norwegian Fishery and Marine Investigations." Many important questions are, however, left to future investigations. The winter stock in the deep waters is thus certainly due to an invasion of specimens who have sunk to great depths in summer and autumn, but the materials are so far quite insufficient for further studies of this phenomenon. As regards the biological part of the mechanism, still more questions are left, questions which will demand more extensive experimental studies than the very few hitherto carried out. What are, for instance, the factors determining the breeding season of the animals? It is an interesting point that, in the arctic species investigated, males and females with eggs in the oviducts are only found in deep water and at the darkest period of the year. In the wide-spread boreal species, *Calanus finmarchicus*, mature individuals have only been observed after the specimens have for some time been exposed to the stronger light intensities of the surface

layers. Possibly light measurements carried out simultaneously with the collectings will give a more complete understanding of these features.

HELLAND-HANSEN'S AND NANSEN'S HYPOTHESIS

Neither in Damas' paper nor in the *Bulletin Trimestriell* (May-June, 1904), where the figures of frequencies of the species in Damas' materials are published, have I been able to find any foundation for Damas' opinion that the spawning of the animals is limited to certain isotherms. Both papers mentioned quite simply show the same phenomenon as just described from the Lofoten area, certain limited areas with high frequencies of certain planktonic species.

It would at present have been very difficult to find any relation between the distribution of Damas' three species and the environment, had it not been that Helland-Hansen and Nansen (1909, pp. 312-316), when they published their discovery of the cyclonic system in the southern Norwegian Sea, had also made the problems raised by Damas the object of a short discussion:

When he wrote his interesting paper, Damas did not know in detail the result of our investigations as regards the movements of the water in the southern Norwegian Sea; he only knew our discovery of the great cyclonic system. He could not therefore give a detailed explanation of the distribution observed. We think that our circulation gives a quite natural explanation. According to Gran's (1902) and Damas's investigations it seems as if there are different species of plankton organisms, *e.g.*, *Calanidae*, that are quite characteristic for certain areas in open sea; within these areas they abound, while they are comparatively rare outside them. We may assume that they are in a way stationary there. We can only explain this fact by assuming that the water masses of those areas are also more or less stationary, and are renewed comparatively slowly. We have found in the southern Norwegian Sea especially three different areas where there is probably a very slow renewal of the water-masses; and where a great part of the water probably remains for a long time, exposed to circulatory movements in various directions.

After having given an account of these areas (which will be seen in Figs. 6 and 7), the two authors continue:

If we now look at Damas's chart (Fig. 6) it is striking how exactly his areas of three kinds of *Calanidae* coincide with the four areas just de-

scribed. *Pseudocalanus* is stationary in the central water chiefly of Atlantic origin, in our central area 1 of the cyclonic system. *Calanus hyperboreus* is stationary in the axis of cold and heavy Arctic water described as our area 3. Damas's boundary line (Fig. 6, B) of the area coincides almost exactly with the equilines mentioned above. *Calanus finmarchicus* is evidently stationary in the mixed waters of Arctic and Atlantic origin, in our areas 2 and 4.

In their paper Helland-Hansen and Nansen have not mentioned any reason why they have compared Damas' chart of the distribution of the species, which Damas regards as surface species, with the chart of the movements

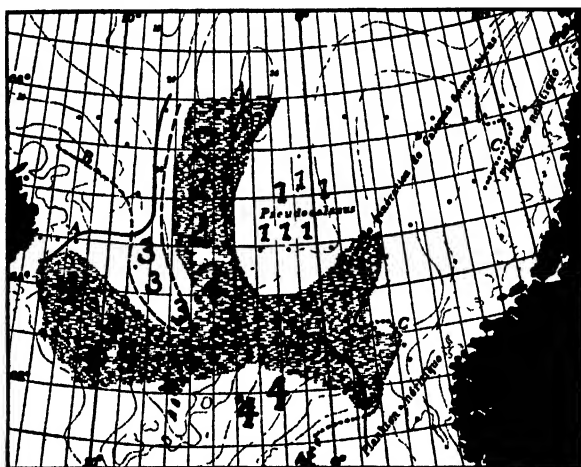


FIG. 6. Distribution of *Calanidae* in the southern Norwegian Sea according to Damas. The lines mark the boundaries of regions where the different species abound: A, adults of *Calanus finmarchicus*; B, *Calanus hyperboreus*; C, Neritic plankton. The broken hatching indicates the distribution of larvae of *Calanus finmarchicus*; the numbers indicate the more still standing water masses at 300 meters (reconstructed from Damas, 1905, Pl. I).

of the water masses at upper 300 meters. As we have seen above from Lofoten, it is only during winter that the species are stationary, and then they are stationary in depths of 300 meters or more, where the water is comparatively slowly renewed. In spring and early summer they are exposed to the swift surface currents and carried far away from their original area. The temperature, and still more the light conditions are, however,

somewhat different in Lofoten and the southern Norwegian Sea. The behavior of the species in the two places therefore are not directly comparable, had it not been that materials from the southern Norwegian coast (Nordgård and Jørgensen (1905), Ruud (1929) and my materials from Oslofjord and Møre), where the conditions are not much different, had already shown the same main features as in Lofoten. No doubt, Helland-Hansen and Nansen were right in choosing just a depth of 300

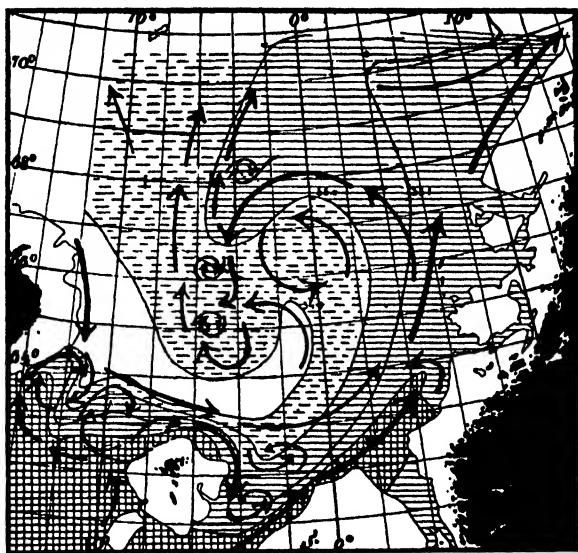


FIG. 7. Direction of currents in upper 300 meters and distribution of salinity at 300 meters in the southern Norwegian Sea (Helland-Hansen and Nansen, 1909).

meters for comparing the biological and hydrographical conditions. Damas' chart shows the species some time after the vertical migration; accordingly they have already been exposed to the effect of the swifter surface currents. The narrower areas where they have previously been stationary accordingly have to be found in places which the surface currents have already passed. It is striking how we, by regarding Helland-Hansen's and Nansen's stationary areas as winter areas for the species

in question, especially as regards the best studied species *Calanus finmarchicus*, can recognize the main features from Lofoten. The species has been carried away just in the direction of the surface currents. In the Shetland-Faeroe ridge, through which the strongest current runs, the species is nearly completely washed away, corresponding to what we have seen from where the current runs into the Lofoten area. Under certain circumstances we may in details follow the effect of the surface current (see Fig. 9) upon the extension of the spawning area. The manifoldness and complexity of these results—each of them reached independently—have now become so great as to make it extremely improbable that this agreement between hydrographical and biological data is fortuitous. No doubt, we have here arrived at a more natural and more well-formed explanation of these phenomena than assumed by Damas in his circulation hypothesis.

GENERAL DISCUSSION

I

Most authors, discussing the factors limiting the geographical distribution of animals, have regarded temperature as a factor of dominant influence. This matter has recently been the object of a brief account by Brown (1929) in this journal, and it should not therefore be necessary to go further into this question here; only a few additional remarks will have to be made.

Regarding marine and limnetic plankton animals and bottom animals with pelagic larvae, many other ecological factors than temperature, especially the light, have, according to the many papers on this subject, an important influence upon physiological processes of animals, and certainly therefore ought to have been given a wider space in discussions dealing with the geographical distribution.

Of great importance for the understanding of the effect of temperature on the distribution are the sugges-

tions made by Hofsten (1915) as regards marine bottom animals, recently strongly supported by the experiments carried out by Runnstrøm (1925, 1927 and 1930). The chief line in the theory of Hofsten is that the temperature limits for the younger stages and the reproduction process are much narrower than the temperature limits at which it is possible for older specimens to live. Runnstrøm has found that the cleavage process of the eggs and the gastrulation can only take place within a limited part of the temperature scale. At temperatures outside this interval these processes show pathological features.

The temperature interval which Runnstrøm found for the normal development of different species could, like the temperature reactions described by Brown, be arranged in certain groups characterized by lower or higher limits. These groups were found to correspond to the different distributional types, arctic boreal (-1° – 11°), boreal (4° – 16°) and Mediterranean boreal (8° – 23°).

Usually Runnstrøm, for the same species, found the same reactions to temperatures at different localities (Norwegian coast, Mediterranean), Brown having arrived at the same conclusions by comparing his own observations from the United States to those of Weismann from Germany. In other cases, Runnstrøm found a discontinuous splitter (*e.g.*, *Mytilus edulis*) of the species, followed by difference in spawning time and horizontal distribution, very much like the difference mentioned by Brown between "clone 984" and the normal *Daphnia pulex*. Accordingly, we can not always regard the morphologically determined species as being physiological units in the geography of animals.

II

In an ocean without any convection current, but with the other ecological factors unchanged, we should surely find the planktonic species arranged in circumpolar belts closely following the limits of temperature and light conditions.

What are now the effects of convection currents?

The Gulf Stream, from which most of the waters of the Norwegian Sea originate, has in the Mexican Gulf and neighboring areas in the upper 100 meters a temperature of between 22° and 28°. When entering the Norwegian Sea its temperature is usually about 9°. Chiefly according to the space of time after its entrance in this area it will further get temperatures between 9° and -2°. Altogether the Gulf Stream thus includes more of the temperature scale than found by Runnstrøm for the normal development of animals from three different regions. In addition to the change in temperature also other ecological factors, like light conditions, light reactions of the animals and nutritive substances, are changed.

The pelagic life of the Gulf Stream is in its origin tropical. The many papers on the distribution of the different species in the Atlantic show us that it is but very few of the Atlantic species that really, together with the water masses, enter the Norwegian Sea and succeed in reproducing there (Hjort and Ruud, 1929, pp. 86). The copepod *Rhincalanus nasutus* represents, for instance, a typical Atlantic species; its limits of distribution into the Norwegian Sea were determined by international investigations 1902-08 (Farran, 1910), only in years with extraordinary strong supply of warm water (Sømme, 1929) some few specimens are brought further north (Figs. 8 and 9).

It here appears to be a typical phenomenon due to changes in the environment along a convection current which is demonstrated, a phenomenon certainly well known to marine biologists. It might possibly be due to mortality along the current, but as we do not know how much may be due to sinking of organisms, deficiency should be preferred as a more adequate name.

The salinities along the Gulf Stream do not show a great deal of variation. This is, however, the case for other convection currents; for instance, the current system of the Baltic and the eastern part of the North Sea.

According to Ekman (1914), the copepod *Limnocalanus grimaldii* (de Guerne) appears to be stationary in the northern and eastern bay of the Baltic, where the waters are almost fresh. The species was here (*l.c.*, pp. 496) found to be present in 97 per cent. of the hauls made. In other parts of the Baltic it was found in 83.3, 71.4, 16.6 and 9.1 per cent. of the hauls, the percentage of occurrence decreasing with an increase of salinity and the distance from the areas where the species was found to be stationary. Ekman's materials thus demonstrate differ-

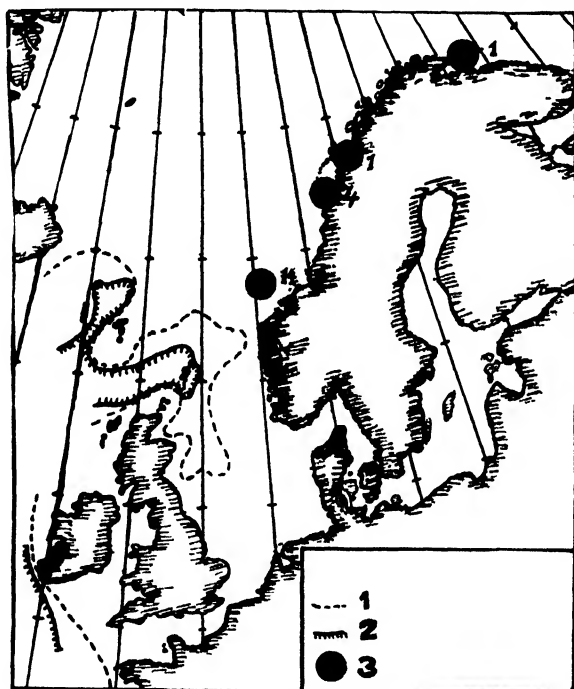


FIG. 8. Distribution of *Rhincalanus nasutus* towards the Norwegian Sea. (After Farran, 1910, and Sømme, 1929.) 1—Extreme limits, 1902-8. 2—Boundary line, 1902-8. 3—Other records of *Rhincalanus nasutus*.

ent stages in deficiency of the species in the convection current running out of the area. Of great interest is further the statement made by Oberg (1906, pp. 63) regarding the oceanic specimen *Paracalanus parvus* Claus. The copepodid and the three later naupliar stages of this

species were frequently found in the western part of the Baltic, while the three earliest stages were always missing. Apparently the species do not succeed in breeding in the waters of low salinity.

While the water masses of a convection current thus appear to be characterized by an increasing deficiency of certain species, the more still-standing water masses of the ocean will contain the species preadapted to the area, species which can live and reproduce there.



FIG. 9. The currents of the Norwegian Sea at 100 meters. (After Helland-Hansen and Nansen, 1909.)

Calanus finmarchicus, this important and characteristic species of the northern seas, is, according to Farran (1920), along the English coast but very scarce outside the isohaline of 35.5 pro mille. Surely this scarce stock, however, is stationary in the northeastern Atlantic (Nordgård and Lysholm, 1915, and others), the water of

the Gulf Stream therefore having already in this place obtained an admixture of this species. An increasing admixture takes place during and after the entrance of the water into the Norwegian Sea (Fig. 9), partly from the areas to the west of the current (Damas, 1905) and partly from the coastal centers and more still-standing water masses on the eastern side. According to Ruud (1929), however, the species is poorly represented in the axis of the current off the southern Norwegian coast. Areas rich in this species are, in the Norwegian Sea, only found along the edge of the continental slope, in the coast waters, and in widely extended areas on the western side. Some of these are the areas described by Damas, others are found further to the north (Nordgård, 1902, Gran, 1902, Damas and Koefoed, 1907, With, 1915, and others). The surface waters of some of these localities here mentioned may sometimes be colored red from the abundance of this species.

For the arctic species, *Calanus hyperboreus*, I have recently got a similar picture by examining the very representative samples from the Danish "Godthåb" expedition in 1928 from the Davis Strait and Baffin's Bay. The water masses entering this extensive arctic area along the Greenland coast are exceedingly poor as regards this species. In the northwestern Baffin's Bay, however, where more still-standing waters are found, generally covered with heavy ice, and therefore not previously entered by expeditions, large numbers were discovered. The numbers caught here are on average 10,000 times as high as in the poorer parts of the area. The current running out of this rich area, following the western side, also showed great numbers, the catches usually being of similar size order.

Regarding *Calanus hyperboreus* the very interesting investigations by Bigelow (1926) from the Gulf of Maine also seem to me to be in striking accordance with these observations. It seems to be in the more still-standing water masses of the western part of the gulf that this

species is stationary during the winter. The current entering the area from the eastern side carries this species into the area during late summer and autumn.

No doubt, in these four areas, the Norwegian Sea, Baffin's Bay, the Lofoten area and the Gulf of Maine, as regards the species mentioned, we meet with a factor in the production of animal plankton hitherto only mentioned by Helland-Hansen and Nansen, namely, the age of the water masses in their biological area. The exact valuation of this new factor must be reserved for future investigations planned and carried out for this purpose. A more intensive study of the distribution also in the winter is, for instance, wanted. Certainly the possible relation between the current system and the production from different areas may be fairly well demonstrated in species like *Calanus finmarchicus* and *Calanus hyperboreus*, where reproduction takes place comparatively slowly, *Calanus finmarchicus* having two, possibly under certain circumstances three, spawning periods a year, *Calanus hyperboreus* having but one very short breeding season and a number of eggs seldom exceeding 400 in each female. It will take many years for these species, in areas where they are scarce, to establish numbers like those which are actually found within the rich areas. Large numbers of these species therefore can thus not be produced in a short time, unless there is a great stationary stock during the winter before reproduction takes place.

Whether other species of planktonic organisms will follow the same rule can not be discussed at present. Judging from the many papers on the distribution of holoplanktonic species, however, and from the instances here treated, it seems to me that the two oceanographers Helland-Hansen and Nansen, whose work in many directions has been of fundamental importance to our present knowledge of the physics of the sea, in their short chapter on the biological consequences of their discoveries, which have been practically buried for 20 years, have

also pointed out main lines for a future biological research of the oceans.

SUMMARY

(1) The distribution of the two species *Calanus finmarchicus* and *Calanus hyperboreus* and the frequency of the species in different areas of the Norwegian coast waters during spring, has been found to depend upon the size of the winter stock in the deep waters of the fjords, and upon the currents carrying the individuals away from the winter areas after the yearly vertical migration of the species.

(2) In the open oceans the more still-standing water masses, as first advanced by Helland-Hansen and Nansen (1909), appear to contain the stationary stock of plankton animals. From these waters the greater masses in the surrounding areas are renewed every spring. The water masses of the convection currents have been found to be characterized by an increasing deficiency of the species along their course, due to changes in ecological factors.

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ABOUT NOMENCLATURE

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BIOLOGICAL nomenclature is neither a science nor an art based on science. Like revelation, it is a prolific source of controversy. About all that has been done is to make rules that resemble creeds and attempt to enforce them by ecclesiastical methods. And it is not binomial but synonymical and is likely to become more so. Binomial relates to a given list or an edition of some work.

I have changed generic names used in 26 earlier papers as follows: Lower Hymenoptera 50, Diptera 33, Lepidoptera 39 and flowers 16. The most confusing changes are such as *Ammophila* to *Sphex* and *Sphex* to *Ammophila*, *Myzine* to *Elis* and *Elis* to *Campsomeris*, *Nuphar* to *Nymphaea* and *Nymphaea* to *Castalia*.

Name-makers are like the native bees. It is quite interesting to observe their habits, but quite absurd to undertake to change them. It took about 40 years for one of them to find this out.

Conditions of changing names: These are priority, erroneous determinations, describing sexes as distinct species, *nomina conservanda*, dividing genera, uniting genera, revising caconyms, restoring caconyms, naming families for typical genera, changing rank of families, subfamilies and superfamilies, changing groups for uniformity of endings. Any one desiring to change names has these for excuses.

Psychology of nomenclators: They show a predilection for making and changing names. It is quite probable that a time when names are changed for a given excuse will be followed by a time when they will be changed for a different excuse. The lumpers and splitters alternate. The nomenclator desires stability, but he wants to put it off until he gets through making changes. One of the most remarkable things about some authors is their

mania for making names and their ignorance of the things to which they assign them.

Erroneous determinations: Some authors make a lot of erroneous determinations, so that names used a while have to be changed. Townsend distinguished many species of Tachinidae. He was followed by a set which suppressed his names as synonyms. A third set restored his names.

Artificial species: Ignoring the fact, and the importance of the fact, that bees have two sexes, these are often described as distinct species by persons who have never seen a respectable local collection, so that half the names must be suppressed when some one who knows the insects comes along. In a recent paper only 4.8 per cent. of the so-called species of Andrenidae were described from both sexes. It is hard to understand how any one could be green enough to describe male and female bumblebees as distinct species, but they are sometimes referred to different genera. Those who describe the sexes of bees as distinct species suppress proposed genera because their ignorance makes it necessary for them to use large genera. The males often show that there are several distinct groups, but the females are so much alike that these makers of artificial species have to refer all the females to one genus and so throw the males in with them. Then they prate of artificial genera. The catalogues list sexes as species 35 years after I have shown that they belong together. My *Andrena platyparia*, with both sexes, has been cited as a synonym of *A. fragilis*, which is not the name of a species but a sex name for the male of *A. integra*.

Beginners mix things: The commonest mistake is mixing species of insects under the same name. The species-maker can not be relied upon to assemble several specimens of the same species, so that a specimen is selected as the type. The descriptions frequently are not real contributions to science, not publications in any true sense. One does not use them to identify species but

goes to some museum to find out what the author described. The species is taken out of the literature and out of the field and localized in some collection. The interests of the museum are made to outweigh those of the science. And it is assumed that one who is not competent to interpret the description is competent to compare the type. It is said that hardly one fourth of 20,000 American insects can be determined from the descriptions. The most incompetent entomologists have their names preserved by the types. Except for this questionable "service," the British Museum has been the greatest stumbling-block in the way of those naming American insects.

Persons who like general terms naturally object to "Zoological Records" or "Botanical Abstracts." "Biological Abstracts" even is not big enough for them. They must mix science with the applications of science, stretching biology to include farming and making potato flour. University must take in everything from the kindergarten to the medical school. With them fever is temperature, a revolver is a gun, and doctor includes everything from savant to medical or religious faker. Professor is "any old thing."

When we have people who can not distinguish science from superstition, scientific demonstration from miracle, mythology from history, research from plagiarism, biology from agriculture, and who think that science and the applications of science are the same thing, it is quite natural that they should mix genera.

Pasteur says: "Few persons comprehend the real origin of the marvels of industry and the wealth of nations. I need no other proof of this than the employment . . . of the erroneous expression *applied science*. It is proof of the imperious necessity of reform in our superior education. There exists no category of sciences to which the name of applied science could be given. *We have science and the applications of science*, which are

united to each other as the fruit and the tree on which it grew."

Under science bunglers include applied science and Christian science. When they intend to make a distinction, they say pure, theoretical or fundamental science. I wonder if the psychologists would include all the fakers as applied psychologists? Applied science often appears to run into pure graft.

Application of scientific method is slow enough. If it is good for anything, it ought to be good for the cure of superstition. But the most effective propagators of superstition are some persons who have gained reputations as authorities in science. So the "learned pate ducks" to the superstitious fool. These people seem to have science without scientific method.

Priority: Persons who make names without regard to priority are often followed by some who are particular about it, so there is a lot of new names. The historically correct names, however, may not have the most important relation to the literature. The friends of priority will have to use these names whether they like them or not. The most effective enemies of priority are the authors who fail to cite the names they propose to replace. Their work is adapted only to beginners who have never used any names.

Persons desiring to make new names favor excuses and ignore reasons, as in changing *Compositae* to *Carduaceae*, *Labiatae* to *Lamiaceae*, *Papilionaceae* to *Fabaceae*, *Pompilidae* to *Psammocharidae*. It is a remarkable fact that authors, very strict about priority of specific names, will deliberately make plans to change family names which are as much entitled to it. And the same persons will show up as plagiaristic crooks when the priority of other persons' observations is involved. One editor who does not distinguish scientific work from school-teaching says that "matters of priority and personal credit are not our primary concern, but rather the advancement of the science in which we choose to labor." He does not

regard personal credit or the history of the subject as of importance in securing the advancement of science. Some editors protect their plagiaristic contributors.

To change names proposed by other authors, considerable juggling is resorted to. I named 6 superfamilies of bees with the ending *oidea*. Another author suppressed these with one hand and appropriated them with the other, changing *oidea* to *formes*. My Colletoidea, with families Prosopididae and Colletidae, was changed to Hylaeidae, with subfamilies Hylaeinae and Colletinae. My Pygidialia, with two superfamilies and ten families, has been suppressed and Andrenidae used instead, the families reduced to subfamilies. And that is the first time Andrenidae has been used in that sense.

Subdividing genera: Since Linnaeus, genera of insects have been subdivided until the insects of New Jersey belong to 3,082 genera, averaging 2.9 species: Hemiptera 2.4, Coleoptera 2.8, Lepidoptera 2.9, Diptera 3.0, Non-aculeata 2.6, Lower aculeata 4.5, bees 7.3. The authors did not have to ask general zoologists, amateurs, outsiders, the public or the deplorers. The New Jersey groups which average more than 2.9 species to the genus evidently have been neglected. As regards genera the students of bees appear to be the most backward. The deplorers lump genera and have their own excuse for changing names.

Subgenera: The history of almost any group will show that these are suppressed genera. Those now recognized generally were proposed as subgenera. The subgenera insisted upon are usually as distinct as the genera recognized. Some authors affect a gnosis which enables them to distinguish them, but they can not tell the difference. I have named three genera, *Xanthosarus*, *Chloralictus*, *Trachandrena*. Authors recognize the distinctions but use circumlocutions: "The *latimanus* group of *Megachile*," "Subgenus *Chloralictus* Robertson," and "*Trachandrena* group." I like one word for a group and let others call it a genus or subgenus.

The so-called subgenera are suppressed genera in another sense. They are made but not used. It is strange that authors will make these categories when they do not intend to use them except by circumlocution or in parentheses. Several authors have suppressed some of my genera, based on structure, as subgenera and then divided them into other subgenera, based on color. A group divisible into subgenera I regard as a genus.

The lumpers are often unable to distinguish genera after their differences are pointed out. The best they can do is to arrange species in the alphabetical order of their names.

I have asserted that beginners prefer large genera. Another author says beginners are especially likely to want to divide genera. My claim is that, while they would like to do this, they are unable to make the distinctions.

That categories have more value the more they include is only an opinion of bunglers. One writer thinks that genera ought to be divided into families, an extreme cranky view. When he suppresses genera, it is not because they are not distinct but because he thinks there are too many.

Some think that subdividing genera increases difficulties of memory. You can remember the names of fifty species belonging to ten genera easier than if they all belong to one. Could you remember peoples' names better, if everybody in town were named Smith? If I call every fabric in a store "cloth," it does not follow that the storekeeper does not need other names.

Some, who are worried about subdividing genera, make the genus synonymous with the family, thus having two names for the same category. If subdividing genera results in one for each species, uniting them results in one for each family.

The most remarkable thing about the lumpers is their affectation of superiority. Anybody can mix species of insects, but it takes an entomologist to separate them.

To mix two species is worse than to make a dozen synonyms of one. In the latter case the records can be united, but in the former all the records have to be rejected because one can not tell to which they apply.

Criteria for genera: Many genera are suppressed because they contain transitional forms. This form is a theological concept correlated with the theory of special creation, and used by persons who do not apply the theory of evolution. It proves that the genera were not created distinct, but it does not show that they should not have separate names. If the taxonomist finds 50 forms quite distinct from another 50, and one transitional between them, he suppresses one of the genera. His classification is based upon the exceptional case. And he refrains from making genera and hesitates to accept those based on a limited fauna, for fear transitional forms will be found.

According to evolution, the members of natural groups are specialized states of the same thing. If a universal search were made, or the past record restored, the members might be reduced to one species. Consequently, the more names you needed, the fewer you would have.

The structural characters of bees have been commonly ignored and they have been described as if they differed only in size and color. So the students of bees favor genera based on color and are indifferent regarding those based on structure.

When the other insects of New Jersey show an average of 2.9 species to the genus, the presumption is pretty well established that the bees ought not to show an average of 7.3 to the genus, and that their generic determinations are erroneous, having been in rather incompetent hands. A writer who evidently thinks that this presumption is of no value says: "The question, how many genera should we recognize, remains in a manner insoluble, or necessarily dependent largely on conventions, which differ at different times and in different groups." Conventions

are the refuge of unintelligent imitators. This author criticizes work in a line which he regards as insoluble.

Nomina conservanda: To protect the nomenclature from what often amounts to the perverse ingenuity of the priority sharps, we have *nomina conservanda*. And they make a mess of another kind. Prior names could be preserved in the record without upsetting names which have a considerable place in the literature.

Revising caconyms: We have things like *confluenta* for *confluens*, *Titusi* for *Titi*, *Anthidiellum*, a Greek diminutive with a Latin diminutive tail, like "little kiddie," and *Brachydiastematotherium*. "Brachy" is put in for shortening. As an excuse for changing names, revising caconyms works both ways. *Bembix* is emended to *Bembex* and then juggled back to *Bembix*.

Mutation: Many make a great fuss if unfamiliar names are used. They want all the owls referred to *Strix* and the hawks to *Falco*. Not only are old genera divided, but it is a poor thing that does not belong to two or three genera. Butterflies flit back and forth between two genera. The name of the chimpanzee is *Pan*, or *Simia*, or *Troglodytes*, or *Anthropopithecus* or *Mimetes*, *niger*. The screech owl is *Strix*, or *Scops*, or *Megascops* or *Otus*, *asio*. The small-mouthed black bass is *Bodianus*, or *Callicurus*, or *Centrarchus*, or *Cichla*, or *Dioplites*, or *Etheostoma*, or *Grystes*, or *Lepomis* or *Micropterus*, *dolomieu*. In the last three cases the stability of the common name makes the rest appear idiotic. The name of a common wasp is *Sphecx*, or *Proterosphex*, or *Ammobia* or *Chlorion*, *ichneumoneus*. I have used three of these names. A bumblebee named by me is *Bombias*, or *Bombus*, or *Bremus*, or *Auricobombus* or *Nevadensibombus*, *auricomus*. For the thistle, I have changed from *Cirsium* to *Cnicus*, to *Carduus*, and back to *Cirsium*. Natural history changes to biology, ecology or bionomics and bio-kinetics. As fast as you adopt one, it changes to the other. So with evolution, speciation, mutation and genetics. Evolution refers to the changes in time of the

same species, or to the origin of specific characters, seldom to the origin of the species themselves. If A uses certain names, B will change them, and C will change back to A's names.

Biological nomenclature a failure: In spite of its universality and quasi-linguistic uniformity, the so-called binomial nomenclature is a failure as a naming system. The identity of the name is destroyed by changing the genus. The binomial degenerates into a quadriminomial like this: *Sphecodes annulatus* (Ill.) D.T. The parenthesis is the tin can on the dog's tail. It signifies that you will not find the original generic names in "Ill.'s" work, whoever "Ill." is. In the minds of pedants the personal matter of the author's name takes precedence over the taxonomic matter of the generic name which is omitted. A trinomial like this would preserve the identity of the name: *Sphecodes Dichroa annulata* Ill. The original binomial could be retained and the genus to which the species is later referred could be prefixed to it. Names of persons could be recorded in nomenclature or synonymical lists, but not used in the literature.

THE GENETICS SOCIETY OF AMERICA

PAPERS READ AT THE ATLANTIC CITY MEETING

PROFESSOR P. W. WHITING, SECRETARY

1. *The Effects of X-rays on the Chromosomes of Circotettix verruculatus (Orthoptera)*. (15 min.) (Lantern): EDWIN R. HELWIG, University of Pennsylvania. (Introduced by C. E. McClung.) When a simple or reciprocal translocation between non-homologous chromosomes has occurred in a spermatogonium the attraction of homologous elements at synapsis results in the union of chromosomes, which are not wholly homologous, and, consequently, compound or multiple chromosomes should be present in the primary spermatocytes. Multiples of this nature are commonly found in individuals of *Circotettix verruculatus* after they have been exposed to x-rays. The multiples are of two types; circles or closed rings, and chains or open rings. The circles are formed as a result of a reciprocal translocation between non-homologous chromosomes. The chains may be formed in several different ways, but most frequently they are the result of the translocation of a section from one chromosome to a non-homologous chromosome. Sometimes they appear to be caused by a reciprocal translocation, in which one of the segments has become attached in an inverted position, thus making synapsis with its homologous section impossible. Other arrangements of the chromatin may be less frequently responsible for the formation of chains. In the anaphase of the first maturation division the configuration of both the circles and chains is usually such that the segregation of homologous elements is complete. However, in a small number of the cells, the structure is such that segregation is only partial.

2. *Chromosome Studies in Stauroderus*. (15 min.) (Lantern): H. IRENE CORY, University of Pennsylvania. (Introduced by Magnhild M. Torvik.) In *Stauroderus scalaris*, an Orthopteron of the subfamily Truxalinae, there are seventeen separate spermatogonial chromosomes, six V-multiples and eleven univalent rods. In the telophase of the last spermatogonial division, each of these chromosomes becomes unusually concentrated at the end which attached to the fiber (proximal end), and these concentrations become fused into a rounded mass, the so-called chromoplast (Eisen, 1900). This body, to which the chromosomes re-

main attached and from which they appear to run out, persists into the early diatene stage of prophase. Before the beginning of peritene, however, the chromoplast separates once more into its component parts, which are recognizable as the proximal ends of the rod- and the proximal portions of the V-chromosomes, and which are given the name "polar granules." They do not diffuse during prophase, but are identifiable throughout chromosome development. In favorably stained material, the first spermatocyte metaphase multiple shows eight separate spindle fibers, one for each chromatid. The accessory is precocious in its behavior and differential in structure at all stages. In first spermatocyte metaphase, it is a non-chromatic vesicle with chromatin granules concentrated around the periphery and a deeply chromatic core. The proximal granule persists as a densely stained mass at the tapering end of the element. The persistence of certain identifiable portions of the chromosomes furnishes a further proof of their individuality and constancy of organization, and it may serve as a new criterion for interpreting chromosome movements.

3. *Studies in Anuran Chromosomes.* (15 min.) (Lantern): EMIL WITSCHI, State University of Iowa. The chromosomic constitution of five American species of toads is the same as that described by several authors for European and Japanese species of the same genus. Haploid sets show 6 large and 5 small chromosomes. *Scaphiopus holbrooki* and *Rana temporaria*, probably also *Hyla crucifer*, have 13 chromosomes, while *Rana arvalis* has only 12. Characteristic features of chromosomes of closely related forms will be described, and the importance of comparative studies in Amphibian chromosomes with respect to the problem of the evolution of the sex chromosome mechanism will be emphasized.

4. *Anatomical Differences between Chromosomal Types in Datura.* (7 min.) (Lantern): E. W. SINNOTT, Columbia University, New York City, and A. F. BLAKESLEE, Carnegie Institution, Cold Spring Harbor, Long Island, New York. Anatomical differences, particularly in the flower stalk, have been studied in the primary and secondary chromosomal types of *Datura stramonium* and in haploid, diploid, triploid and tetraploid plants of this species. Specific differences have been found in the size, shape

and number of cells in various tissues; in the relative development of epidermis, fundamental tissue, pericycle, internal and external phloem, cambial zone and xylem; in the number and shape of the vascular bundles; in the size of starch grains, and in other respects. In general the condition of the diploid plant was found to represent a balance between the various primary types, and each primary to be intermediate in its various characters between its two secondaries. Exceptions in each case were observed. Differences between members of the polyploid series are due chiefly to differences in cell size, but this was not found to occur in all tissues. Similar anatomical differences were found in the tissues of root, stem, petiole, capsule stalk and floral organs. It is concluded that internal structures are no less affected by genic differences than are external ones.

5. *Evidence against the Occurrence of Crossing-over between Sister Chromatids.* (15 min.): H. J. MULLER, University of Texas, Austin, Texas, and ALEXANDER WEINSTEIN, Johns Hopkins University. To test whether unequal crossing-over occurs between sister strands in a region far removed from the spindle fiber, X-chromosomes containing inversions, including the Bar region, were obtained by irradiation (winter, 1928-1929). Inviability and infertility rendered these unsuitable; but the scute-8 inversion (discovered by Sidoroff, further analyzed by Serebrovsky and Levit) has provided the necessary material. Females of the composition $\frac{sc^8 \ fu \ B \ f \ w^a}{sc^8 \ B \ w^a}$ have been made up and have so far yielded 21 offspring containing round or double-Bar chromosomes derived from the mother. All of these were also cross-overs between forked and fused; hence interchange was always between homologous chromosomes, whereas on a random basis one third of the cases should have been crossovers between sister strands. These results show that the absence of sister-strand crossing over in ordinary Bar races is not due to the proximity of the spindle fiber, despite the fact that the spindle fiber is known to affect crossing over in its neighborhood (Offermann, Stone, Muller, 1931, 1932; Beadle, 1932); for the data of Offermann and Muller show that in the scute-8 inversion Bar is outside the sphere of influence of the spindle fiber. While the special nature of unequal crossing over does not permit of completely unqualified generalization to ordinary crossing over, the

results of the present experiment are in agreement with those based on mathematical methods (Weinstein, 1928, 1929, 1932) which show that if sister strands are completely separated from each other before crossing over, interchange between them is (except on special assumptions) inconsistent with the observed crossover frequencies.

6. *Crossing over in Triploids and in Attached-X Diploids.* (15 min.): ALEXANDER WEINSTEIN, Johns Hopkins University. The formula for multiple-strand crossing over (Weinstein, 1928, 1929, 1932) when applied to triploids yields (with random recurrence and either with or without sister-strand crossing over) class frequencies which are impossible (greater than the total, or negative). This shows (in agreement with Muller's unpublished analysis, but contrary to other interpretations) that synapsis in a triploid does not involve all three chromosomes equally throughout their length. This is further borne out by the non-alteration of crossing over when one chromosome in a triploid contains an inversion preventing crossing over. In diploids with attached X's, if the frequencies of tetrads are calculated by the multiple-strand formula, the types of offspring can be deduced from the following considerations. (1) If both attached pairs are heterozygous at a given level, the next distal crossing over results in homozygosis distal to itself only if the interchange is between unattached homologous strands. (2) If both attached pairs are homozygous at a given level, the next distal crossing over results in homozygosis distal to itself only if the interchange is between sister strands. The experimental frequencies agree with those calculated on the basis of random recurrence without sister-strand crossing over. Sister-strand crossing-over would result in discrepancies; *e.g.*, the frequency of homozygosis for any recessive gene could never exceed one sixth. Random recurrence, including interference of crossing over between two strands with crossing over between two others, can be explained if the halves of a split chromosome are not entirely separate until after crossing over. The absence of crossing over between sister strands can also be explained if each split chromosome is at first a string of double genes rather than two strings of single genes.

7. *Double Strand Crossing-over in Zea mays.* (15 min.) (Lantern): MARCUS M. RHOADES, Cornell University. The occurrence of double strand crossing-over has been genetically demonstrated

in *Drosophila*, *Habrobracon* and *Zea*. The demonstration in maize was achieved through the use of plants trisomic for the $pr-v_2$ chromosome. Trisomic plants of $Pr V_2$ constitution were

$$\frac{Pr V_2}{pr v_2}$$

pollinated by disomic double recessive individuals. Among the progeny of such crosses there resulted trisomic individuals homozygous for pr or v_2 or for both genes. The occurrence of these recessive trisomic types is proof that crossing over occurred at a stage when each chromosome was divided into two chromatids. In a total of approximately 4,900 plants there were 62 trisomic plants homozygous for v_2 and 21 which were homozygous for pr . The relative frequencies of trisomic individuals homozygous for v_2 and pr indicates that the locus of v_2 is approximately three times as far from the insertion region as is the locus of pr . The genotypic constitution of some of the recessive trisomic types suggests that the loci of pr and v_2 are on opposite sides of the insertion region.

8. *Stumpy Mosaic of Habrobracon and their Bearing on Interaction and Reduction.* (15 min.) (Lantern): RAYMOND J. GREB and P. W. WHITING, University of Pittsburgh. Stumpy is characterized by lack of tarsal segments, but some specimens showing fluctuation toward wild-type (5 tarsal segments) have one to three segments in one or more tarsi. Females heterozygous for various traits occasionally produce a mosaic male from binucleate egg with post-reduction. Males mosaic for various traits from mothers heterozygous for stumpy and for other traits total 119, of which 60 are non-mosaic for stumpy (32 with all tarsi wild-type, 16 with all tarsi typical stumpy, 12 with tarsi slightly atypical stumpy) and 59 are probably mosaic for stumpy, 53 being obviously mosaic with some wild-type, some stumpy tarsi, while 6 have some tarsi stumpy and some with four or five irregular segments (modified wild-type?). Approximate equality of mosaics and non-mosaics as regards stumpy among the total number of mosaics indicates that in binucleate eggs post-reduction and pre-reduction occur with equal frequency. Morphological and statistical analysis of the mosaics indicates prevailing tendency for right half of body to be genetically different from left and for mutual modifying influence between wild-type and stumpy tissue.

9. *Genetic Evidence for Attraction of Dissimilar Chromosomes in Tetraploid Species Hybrids of Tomatoes.* (12 min.) (Lantern): E. W. LINDSTROM, Iowa State College. From two homozygous but very different tomato species, tetraploid hybrids were produced asexually by the decapitation-callus method. In such material two chromosomes in each tetravalent set are identical genetically and are unlike the two identical chromosomes from the other species. Since these chromosomes eventually emerge as bivalents at diakinesis, it might be expected that like chromosomes (or like parts of chromosomes in case crossing-over occurred earlier and permitted formation of bivalents) would pair preferentially. Such was not the case in four tested chromosome sets as judged by the F_2 and F_3 progenies from the tetraploid F_1 species hybrid. Moreover, the percentages of recessives cropping out were higher than expected on a random assortment of four chromosomes. This points to a pairing of unlike chromosomes in such a tetraploid, and indicates that something more than the attractions of similar Mendelian genes in this cross are involved in chromosome pairing. The formation of bivalents in these tetraploids would seem to exclude typical tetraploid crossing over on Darlington's chiasma hypothesis.

10. *Electrostatic Radiation and Genetic Effects.* (15 min.): F. O. SCHMITT, Washington University, St. Louis, and C. P. OLIVER, University of Minnesota. *Drosophila* males, curly, star, dichaete, were treated between two electrodes twenty-three mm apart, a coil intensity of three amperes, plate of forty-one ma, wave-length two hundred meters. A total frequency of six million cycles per second was given for thirty seconds. Matings were such that any visible or sex-linked, lethal mutation or translocation involving X, II, III could be detected. Only sixty-eight per cent. of the treated males recovered sufficiently to mate, and only thirty-five per cent. of these were fertile. Controls were eighty-two per cent. fertile. The difference is significant. Treated series produced an average of six offspring per fertile, treated male. This corresponds to a t8 dosage (about 3,000 r) in x-radiation. Should the genetic effect be similar, treatment should produce about ten per cent. lethal mutations and an equal frequency of translocations. Control series produced an average of eighty offspring per male parent. Of 2352 F_1 from treated parents, 1795 were fertile and five (possibly seven) lethals occurred; of 1293 con-

trols, 1021 were fertile, and three lethals occurred. In both series no visible mutations or translocations occurred. If seven is taken as the frequency for the treated, the difference between the treated and control series is not significant. The electrostatic field intensity decreased fertility equivalent to a strong x-ray dosage but did not under the conditions here used increase the frequency of mutations or translocations.

11. *On Eversporting as a Function of the Y Chromosome in Drosophila melanogaster.* (15 min.) (Lantern): J. W. GOWEN and E. H. GAY, Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, New Jersey. Eversporting eye color has appeared in several x-ray induced mottled eye stocks. Mottled flies, mated to white, ordinarily produce half white and half mottled offspring, but occasionally we find apparent reversions to normal red eye color. Mated to white, these red-eyed individuals produce progeny about one half of which are white, one fourth mottled, and one fourth red-eyed. Of these three types, mated to white, the white have only white progeny, the mottled breed like typical mottled flies, and the red breed like their red parents. In other words either mottled or red flies, mated to white, have white, mottled, and red-eyed progeny in quite predictable proportions. By cytological and genetic evidence we have proved that mottled and red-eyed flies are genotypically identical, except that the red flies carry an extra Y chromosome, their sex chromosome constitution being XXY in females and XYY in males. Therefore reversion to red eye color in a culture of mottled stock occurs as a consequence of primary nondisjunction; and continued appearance of red eyes in descendants of red-eyed flies occurs as a consequence of secondary nondisjunction. Cytological study of ovaries of red-eyed exceptions reveals the presence of an extra chromosome in each division figure. Genetic proof that the extra chromosome is a Y is obtained by using a factor, bobbed, whose expression is inhibited by the presence of a Y chromosome.

12. *Miniature-5, a New Unstable Gene in Drosophila virilis.* (10 min.) (Lantern): M. DEMEREC, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. A single miniature male was unexpectedly found among 59 offspring of a pair mating. In addition to being miniature the male was garnet, rounded and heterozygous for short vein as expected of flies from that mating.

Since miniature, garnet, rounded flies were not present in the laboratory the miniature male could not have come accidentally into the culture but it belonged to the fraternity in which it was found. The miniature character of that male, therefore, originated through a mutation from wild-type to miniature which occurred in the female parent. Genetic tests showed that the new miniature (miniature-5) is an allelomorph of the previously known miniatures, and that it is unstable, frequently reverting to the wild-type. Two unstable allelomorphs are, therefore, known in the miniature locus, *viz.*: miniature-3 and miniature-5. They are phenotypically identical. The possibility of their being identical genes is indicated by the fact that in both of them alpha and beta forms are found. Gamma form, however, is not yet known in miniature-5. The known forms of both genes behave similarly toward modifying factors which stimulate the instability.

13. *Gene Number, Kind, and Size in Drosophila.* (15 min.) (Lantern): JOHN W. GOWEN and E. H. GAY, Rockefeller Institute for Medical Research, Princeton, New Jersey. Adult males of *Drosophila melanogaster* were exposed to mono-chromatic x-rays from copper and chromium for eight different durations of exposure. Quantitative data were collected on decline in progeny rate, change in sex ratio, recessive lethal sex-linked mutations, and visible mutations. The visible mutations obtained were compared with previously known mutations to determine their possible identity. From analysis of these data we estimate a minimum of 14,380 loci which indicates an average size per gene of 1×10^{-18} cm³. This size is sufficient to contain fifteen protein molecules.

14. *Studies on Factor Interaction in Drosophila virilis.* (10 min.) (Lantern): G. A. LEBEDEFF, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. The 5th chromosome recessive gene ruffled (ru) which causes curled bristles and hairs on the thorax, as has been shown by the author, becomes incompletely dominant in crosses in which the second chromosome dominant rounded wings (R) gene is present. The change in dominance is accompanied by an interaction of the genes, resulting in a new wing character—roofed. When the ruffled gene is homozygous, the effect of both genes is exaggerated: bristles and hairs on the thorax are extremely curled, thorax

has semi-vortex protrusions, wings are extremely roofed, spread and shortened. Further studies show that ruffled gene also interacts with the 4th chromosome dominant gene—clipped wings (Cl). The +/ru Cl/+ flies have slightly ruffled bristles and semi-vortex thorax. Cl- ru/ru flies are extremely ruffled and have well-developed vortex protrusions. The wings are not affected. When rounded and clipped genes are brought together they also interact with each other. R/+ Cl/+ flies are extremely rounded, ruffled, roofed, as the ru/ru R- flies, and have exaggerated vortex protrusions as the ru/ru Cl- flies. This mutual interrelationship of three genes will make it possible to trace the rôle played by each gene in this interaction.

15. *On the Action of Certain Modifying Genes in Mice.* (15 min.) (Lantern): L. C. DUNN and D. R. CHARLES, Columbia University. White-spotted housemice, known to fanciers as piebald, differ from wild type in a gene *s* but vary from almost solid-colored (self) to solid-white with dark eyes. Inbred lines showing no pigment in coat ("all white") were isolated by selection and crossed with other constant piebald lines showing small amounts of white spotting. F₂ and backcross generations showed segregation of several genes with quantitative effects on spotting, independent of and not allelomorphic with *s* ("modifiers"). Analysis of modifiers was made by crossing "all-white" (*ss*) with several constant self-colored lines (*SS*). By continued back-crossing of such heterozygotes to "all-white," the light "modifiers" were accumulated in *Ss* animals (which showed an average of about 40 per cent. dorsal white in the fifth back-cross), and then in extracted *SS* animals which proved to be white-spotted (5-25 per cent. dorsal white). This shows that the "modifiers" act when the main gene *s* is not present and are thus independent spotting genes acting cumulatively with *s*; and that variations in piebald are probably not due to reverse somatic mutations of *s* to *S*, since they occur in absence of *s*. Some of the piebald "modifiers" act also on variegated spotting (*WwSS*). Finally it is shown that yellow (*A^Y*) also modifies both piebald and variegated, acting in both cases to reduce the amount of white. It is concluded that spotting modifiers are non-specific and bring about general quantitative changes in the developmental background against which the "main" genes act.

16. *Two Modes of Developmental Control by Shape Genes.* (8 min.) (Lantern): E. W. SINNOTT, Columbia University. In the developing fruit of *Cucurbita*, from very small floral primordia to maturity, length plotted against width (logarithmically for each) produces a straight line. The lines for different shape types (elongate, sphere, or disk) have essentially the same slope but differ markedly in level. In Huxley's formula $y = bx^k$, k here is much the same for these shape types but b is very different. The relative growth rates for length and width are thus the same from the earliest stages, and shape differences are not due to differences in relative growth rate but to the very early establishment of a developmental relationship which persists. *Capsicum* and *Vigna*, however, show another type of shape development. Here fruits differing genetically in shape show very different slopes of their length-width lines (values of k) and these lines converge at the young primordia. Here the shape types are quite indistinguishable in the primordia but become established through different relative growth rates of the two dimensions. Since in both *Cucurbita* and *Capsicum* fruit shape has been shown to be inherited, it is suggested that the mode of operation of shape genes is different in the two groups. In the former, these genes control differentiation at a very early stage; in the latter they are ineffective at first but operate by controlling relative growth rates throughout development.

17. *Multiple Allelomorphs in Salpiglossis.* (10 min.) (Lantern): E. E. DALE, Union College, Schenectady, New York. A series of true-breeding, modified flower types has appeared in *Salpiglossis* cultures grown from commercial seed. All types involve a modification of the corolla. There are four types designated (a) appendaged limb, (b) cut, (c) notched-1, (d) notched-2. In crosses with normal, F_1 is normal and F_2 shows monohybrid segregation. Crosses between any two members of the series give an F_1 more or less intermediate between the parents except that notched-1 is dominant to notched-2. Thus a single multiple series is indicated.

18. *The Growth Curves of the Length of the Vestigial Wings of D. melanogaster.* (15 min.) (Lantern): M. H. HARNLY, New York University. The growth curves of the vestigial wings of *D. melanogaster* have been determined at four temperatures. They were found to be sigmoid curves for the male at 30°, 31°,

and 32° C. The growth period extended from the sixtieth to the eightieth hour of development as indicated by the curve at 30° C. The curve commenced rising at the same time at 31° but it had a sharper slope and continued to the one hundred and twelfth hour of development. The only change at 32° C. was a steeper slope, the growth period being the same. The growth period and curve for the female was found to be approximately the same as that for the male at 30° but at 31° C., after paralleling the curve for the male from the sixtieth hour of development, growth suddenly ceased at the eightieth hour of development. In the females reared at 32° C. growth of the wings continued beyond the eightieth hour to the one hundred and thirty second hour, the curve being linear. The curves over the period examined at 33° C. (72–120 hrs.) of both males and females were linear and had the same slope as the curve for females raised at 32° C.; only the intercept varied slightly for the three lines. The curves furnish an explanation of the sexual dimorphism shown by this mutant at 30°, 31°, and 32° C. The wings of the two sexes were equivalent at 33° C.

19. *Mendelian Methods Applied to the Ciliate Protozoan, Paramecium aurelia.* (15 min.) (Lantern): T. M. SONNEBORN, Johns Hopkins University. Development of a method of inducing conjugation between individuals of known diverse genetic constitutions made possible for the first time in ciliate Protozoa a study of cross-breeding and segregation. The individuals of clones A and Z, known to differ greatly in fission rate and size, were crossed. The two hybrids descended from each pair of cross-conjugants at first differed markedly in fission rate and size, but less so than did their parents. Later, these differences between the two hybrids of each pair largely disappeared. However, different pairs of hybrids attained different definitive characters, e.g., both members of one pair resembled clone A; both members of another pair resembled clone Z; both members of another pair were intermediate. An F₂ generation, obtained by inducing self-fertilization in one of the F₁ hybrids, yielded segregation of both parental types and of intermediates. Such segregation did not occur in the control group obtained by inducing self-fertilization in the parent clone. F₃ generations, obtained by selfing different F₂ clones, indicated that some of the F₂ segregates were still "heterozygous," others "homozygous" for the clonal characters.

20. *Long-continued Parthenogenesis and the Accumulation of Recessive Mutations in Cladocera.* (15 min.) (Lantern): A. M. BANTA and THELMA R. WOOD, Brown University and Carnegie Institution. In diploid parthenogenesis all the descendants of an individual should be genetically identical except as mutations occur. Prevailing uniformity of the numbers of a cladoceran clone is in accord with this conception. A few morphological mutations have appeared in *Daphnia longispina* during parthenogenesis. In breeding experiments with sexual reproduction (by which three of these mutations have been shown to be dominant single-factor characters, though influenced by modifying factors) an unanticipated finding has appeared—evidence that during parthenogenesis recessive lethal or sub-lethal mutations occur, accumulate in latent condition with continued parthenogenesis, and become evident only in the results of inbreeding in biparental reproduction. Clones of *Daphnia longispina* inbred early in their parthenogenetic history produce sexual eggs which hatch relatively well and the hatched individuals (while not identical in their physiological characteristics) are generally vigorous vegetatively and in parthenogenetic reproduction. On the other hand the same clone, when inbred after a more prolonged period of parthenogenesis (even though it shows no change in the characteristics of its parthenogenetic young) shows reduced hatchability of the sexual eggs, and the sexually-produced young include many non-viable, viable but sterile (produce no parthenogenetic young), slightly fertile, and fully fertile individuals, which, if capable of parthenogenetic reproduction, give rise to individual clones like themselves. When, however, a clone long-parthenogenetic is cross-bred to an unrelated clone, the sexually produced offspring fail to show the phenomena just mentioned. The results are held to indicate the occurrence of and accumulation of recessive sub-lethal mutations during continued parthenogenesis.

21. *Genetic Factors in Relation to the Etiology of Malignant Tumors.* (15 min.) (Lantern): M. R. CURTIS, W. F. DUNNING, F. D. BULLOCK, Institute of Cancer Research, Columbia University. Marked strain and family differences were observed in the occurrence of malignant liver tumors (sarcomata) arising in the walls of cysts of Cestode larvae (*Cysticercus fasciolaris*) when domestic rats from several independent sources, and sev-

eral generations of their pedigreed descendents were experimentally infested with the parasite. In spite of these differences "non-cancerous" infested descendants of a "cancer pair" frequently survived by a wide margin the minimum duration of infestation observed in the tumor bearers, and individuals which developed tumors arose from crosses involving a "cancerous" and an infested "non-cancerous" parent of a "non-cancerous" family indicating that susceptibility is not a single factor Mendelian recessive or that the individuals were insufficiently tested. Families which had few or no tumor bearers among the rats which survived the minimum infestation period showed also the highest percentage of early recoveries from the *Cysticercus* disease. A negative correlation was observed in the tumor bearers between the duration of infestation and the number of the parasites in the liver. Also the percentage of tumor bearers increased directly with the number of parasitic cysts. Further longevity may raise the proportions of tumor bearers in a strain of low susceptibility to *Cysticercus* disease to equal that of shorter lived strains of higher susceptibility. These and other facts shown by a study of the morphology and biology of these tumors indicate that the genetic factors concerned govern the susceptibility to the *Cysticercus* disease and that the malignant complication may arise by a process analogous to somatic mutation.

22. *On the Genetic Basis of Constitution as it Affects Disease Resistance.* (15 min.) (Lantern): JOHN W. GOWEN and RALPH G. SCHOTT, Rockefeller Institute for Medical Research, Princeton, New Jersey. Genetic constitution for disease resistance could conceivably be a single character which enabled the organism to resist a multiplicity of environmental agents or a composite of many independent characters each of which is capable of causing the individual to resist one environmental agent. Study of the survival rates of our strains of mice to a neurotropic virus, pseudo-rabies, shows that the S line has a survival rate of only 8.4 per cent. whereas the sil line rate is 52.2 per cent., the F_1 rate is 23.7 per cent. Previous study of these lines by Schott for survival to *Salmonella aertrycke* showed rates of 75.3 per cent. for the S line, 0.0 for the sil line and 62.6 for the F_1 . The genetic complex which was favorable to survival in one strain favors susceptibility in the other. Selection of resistance

to one agent within this population leads directly to susceptibility to another. The data suggest that genetic constitution is a composite made up of distinct characters some of which favor resistance to one environmental agent, others another.

23. *Linkage Studies in Maize.* (15 min.) (Lantern): WILLIAM H. EYSTER, Bucknell University, Lewisburg, Pa. (A) New genes in chromosome IX. The loci in the chromosome and the somatic expression of genes for auricle stripe₁, aurea₁, aurea₂, dilute aleurone, vivipary₄, crinkly leaves₂, golden plant₃, pale green seedling_{IX}, and small kernel_{IX} are given. (B) New genes in chromosome V. The loci and the somatic expression of genes for brown midrib₁, tinged plant, oil yellow chlorophyll, dwarf_V, vivipary₂, male sterile_V, stiff leaves, pale green seedlings_V, luteus_V, auricle stripe_V, and lemon endosperm are given.

24. *Linkage Relations between Dominant White Endosperm and Glossy Seedling, gl₁, in Maize.* (15 min.): H. K. HAYES, Cornell University. The dominant white endosperm used in these crosses proved to be homozygous WhWh YY. In crosses with colorless endosperm, yy gl₁gl₁, the immediate result was a creamy or pale yellow endosperm color. F₂ segregated for dominant white, recessive white and yellow, although the classes could not be differentiated by inspection in all cases. There was an indication of a possible linkage in the repulsion phase between Wh and gl₁. In studies of linkage relations between dominant white with a mutant gene the crossover gametes can be determined from crosses of the F₁ with a yellow endosperm strain homozygous for the mutant factor. To test the relation of Wh and gl₁ and F₁ (WhWh YY Gl₁Gl₁ × whwh yy gl₁gl₁) was crossed with YY gl₁gl₁. All ears of the backcross indicated a similar relation and the data have been combined. From a total of 1359 seedlings there were 525 new combinations or a C. O. percentage of 38.6 ± 0.9.

25. *Sex-Linked Factors in Crosses between Varieties, Species and Genera of Mexican Fishes.* (15 min.) (Lantern): MYRON GORDON, Cornell University. There are three sex-linked factors in the common platyfish (*Platypoecilus maculatus*): Sp, large spots; N, (nigra) black band; R, red body. Bellamy refers these to an allelomorphic series while Fraser and Gordon postulate close linkage. New data support the latter interpretation.

Females (**WZ**) have been built up containing all three dominant sex-linked factors: *Sp* and *R* in the **W** chromosome, *N* in the **Z** chromosome. In back-crosses to triple recessive males, nearly all the daughters are Spotted and Red while the sons are *N*, black banded. A few cross-overs have occurred which indicates frequency of crossing over between **W** and **Z** chromosomes about one per cent. No abnormal conditions of pigmentation or sex intergrades were detected. *P. maculatus* containing *Sp* and *R* in **W** chromosomes crossed with *P. couchianus* give melanotic daughters; sons are like father (intermediate for size). *P. maculatus* containing *Sp* and *R* in **Z** chromosomes crossed with *P. couchianus* gives melanotic sons; daughters are like father. *P. maculatus* containing *Sp* and *R* in **W** chromosomes crossed with *P. xiphidium* and also with *P. variatus* give melanotic daughters; sons are like the fathers (size appropriate to the cross). *P. maculatus* containing *Sp* alone in **W** and **Z** chromosomes crossed with *Xiphophorus hellerii* produce both daughters and sons with melanotic neoplastic overgrowths of a greater intensity than when *Sp* is present with *R*. There is no evidence as yet for intersexes. Sex chromosomes apparently dominate sex determining influences in this group.

26. *Oxygen Consumption of Different Genetic Types of Goldfish.* (10 min.) (Lantern): H. B. GOODRICH, C. B. CRAMPTON, D. A. WILSON, Wesleyan University, Middletown, Conn. Measurements have been made of the rate of oxygen consumption of different types of goldfish. The selection of fish was based on the genetic analyses made by Chen '28 and Berndt (28) which indicated that the common shubunkin or calicoe fish (TT') is the hybrid between the ordinary goldfish TT and the transparent shubunkin T'T'. The results show that the oxygen consumption of the hybrid is significantly lower than that of either homozygous parental type. If the oxygen consumption of the goldfish TT is taken as unity, that of the transparent shubunkin T'T' is 96.2 per cent. and the hybrid or TT' is only 59.1 per cent. of that of the goldfish. These ratios are based on determinations so far made in weight class of from 6 grams to 20 grams. The oxygen consumption of smaller and younger fish is relatively higher but a similar ratio is maintained. Determinations were made by the Rideal Stewart modification of the Winkler method.

27. *Hereditary Skeletal Variation in the Rabbit.* (10 min.) (Lantern): P. B. SAWIN, Brown University, and Bussey Institution. The rabbit is normally considered to have twelve pairs of ribs. Specimens recently dissected by the class in comparative anatomy at Brown University show an abnormal thirteenth pair. The pedigree of these animals traces to a race being bred at the Bussey Institution. Some idea of the frequency and genetic distribution of this variation has been obtained by dissection and x-ray of more than 1,000 animals belonging to six unrelated races. It occurs in four of these races and has been traced in one case through four generations. The extent of the abnormality varies from a rudimentary thirteenth rib on one side only, to fully developed extra ribs. It may or may not include an extra vertebra inserted between the cervical vertebra and the sacrum. The number of caudal vertebrae is also variable but this is not correlated with the presacral variation. In two lines (E and M), both descended from the same male ancestor (116), regular Mendelian segregation of extra ribs is apparent. Abnormal individuals mated *inter se* have produced 49 abnormal and 17 normal offspring. Abnormals mated to normals have produced 108 abnormal and 100 normal young. Extracted normals breed true. The inheritance of this variation in lines D and N, both of which also originate from male 116, is irregular. Both inbred abnormal and back-cross matings show a much higher incidence of normal offspring, probably due to the presence of modifying factors.

28. *Natural Polymorphism in Related Species of Mexican Fishes:* MYRON GORDON, Cornell University. As a result of two ichthyological expeditions into Mexico (1930, 1932), an analysis of natural polymorphism has been made for all species of *Xiphophorus* and *Platypoecilus* (Tribe *Xiphophorini*). Most variable is *P. maculatus* (Rio Papaloapan System) which has 40 phenotypes in 169 specimens studied. Previous genetic work indicates five genotypes with the probability that five more are to be considered. *P. variatus* (Rio Panuco System) has ten phenotypes, each of which is represented in *P. maculatus*, but with slight morphological differences. *P. xiphidium* (Rio Sota la Marina System) has eight phenotypes, each of which is represented in other species but again with modifications. *P. couchianus* (Rio Grande System) has but two phenotypes out of 500

examined. *X. hellerii* has three subspecies: *hellerii* (Rio San Francisco), *strigatus* (Rio Papaloapan), *güntheri* (Rio Coatzacoalcas). Taken together there are four phenotypes, each of which bears morphologic relationship to those in *Platypoecilus*. *X. montezumae* (Rio Panuco System) has two phenotypes.

Living specimens have been transported to Ithaca, N. Y., for genetic analysis. All the species (except *X. montezumae*) have already bred under aquarium conditions and the patterns are constant. Hybridization is possible specifically and generically. Charts will be exhibited showing polymorphism for all, together with a map of northeastern Mexico showing their geographic distribution. Genetic interpretation is submitted for the polymorphism displayed.

29. *The Time Curve of Facet-Determination in Ultra-Bar Females of Drosophila*: A. H. HERSH, Western Reserve University. In the bar series of *Drosophila*, flies emerging from larvae transferred from one temperature to another before the onset of the determination period have the facet number characteristic for the temperature to which they are transferred. If the transfer is made after the end of the determination period, the facet number is then characteristic for the temperature from which they are transferred. If the transfer is made during the effective period some intermediate number of facets results. By a dissection of the data obtained by Driver on the effective period (1931, *Jour. Exp. Zool.*, 59: 1-28) it has been found that a sigmoid curve of the autocatalytic type fits the data on ultra-bar females. In differential form $\frac{dx}{dt} = kx(A - x)$ in which x is the number of facets at any time (t), A is the maximum number of facets produced by the average individual, and k is the specific velocity-constant. The values of k follow: 15°, 0.0013; 17°, 0.0024; 20°, 0.0033; 22°, 0.0074; 25°, 0.0083; 27°, 0.0127. k in its relation to temperature apparently does not conform to the well-known Arrhenius equation. If the sigmoid curve represents a true first approximation, then differences between members of the bar series in regard to the process of facet-determination do not represent merely a termination of the process at some greater or lesser distance from a common upper asymptote, but that the termination of the process in each case is approached asymptotically, similar to growth processes in general.

30. *Meiosis in Aphids*: E. C. JEFFREY, Harvard University. The author has investigated a large number of aphids during the past five years and has found that the general conclusions reached by Morgan are not justified by the facts. In *Aphis salicicola*, for example, there are eight somatic chromosomes and four reproductive. The primary spermatocyte divides into two equal halves, each of which takes four chromosomes. There is no abortion of the secondary spermatocytes. These in turn again divide into two equal halves with four chromosomes. *Aphis populifoliae* is a common form on the gray birch of New England. Here the somatic chromosomes are sixteen in number and the reproductive are eight. The primary spermatocyte divides, giving rise to two secondaries, each of which contains eight chromosomes. The secondary spermatocytes also divide equally with eight chromosomes. It should be noted that in these cases and in others there are lagging chromosomes precisely as in the other aphids described here and abroad. The lagging chromosomes would seem to indicate the existence of hybridism. Competent investigators on the botanical side have assumed hybridism as the universal cause of parthenogenesis in the higher plants and that would seem to be the natural explanation of parthenogenesis in the Aphids. The Morgan hypothesis that the abortion of one of the secondary spermatocytes, resulting in the formation of only female producing sperms, accordingly appears to fall to the ground because in the form investigated by him the abortion of spermatocytes was merely an indication of hybrid sterility and has no bearing on the question of the production of the parthenogenetic females, as is clearly shown by the cases cited in the beginning of this abstract where parthenogenetic females make their appearance without any abortion whatever of sperms.

31. *Chromosome Rings and Strings and their Significance*: E. C. JEFFREY, Harvard University. Chromosome rings have been shown to be present in a wide range of plants in recent years and there is growing evidence that they have some relation to previous interspecific crossing. Outstanding cases are presented by *Oenothera* and certain of the *Tradescantiae*. The general interpretation has been that the rings of chromosomes or strings of chromosomes are sequences of univalents. An investigation of forms more favorable for cytological purposes than are the *Oenotheras* has shown that the univalent conception of

the rings and strings of chromosomes can not be accepted. In *Tradescantia virginiana*, for example, which is particularly favorable on account of the large size of its chromosomes, the synaptic mates are regularly formed as in such classic types as *Allium cepa* and *Vicia faba*. There is no question whatever of univalent chromosomes in this species. Nevertheless it has rings or strings of chromosomes precisely as in *Oenothera* and other well-known cases. It follows that the genetical conclusions drawn from the presence of rings of chromosomes or strings of chromosomes are without sound cytological basis.

32. *Inheritance of Melanism in the Grasshopper, Melanoplus differentialis*: ROBERT L. KING, State University of Iowa. A melanic form of *Melanoplus differentialis* is rather infrequently found in collections of that species. Breeding experiments show this black form to differ from the usual greenish one by the homozygous presence of a single recessive factor which is not sex-linked.

33. *Genetic Analysis of Parthenogenesis in Nabours' Grouse Locusts*: ROBERT L. KING and ELEANOR H. SLIFER, State University of Iowa. If diploid parthenogenesis in the grouse locusts is the result of the suppression of the second maturation division a genetic analysis of synapsis and maturation may be made. The eggs of females, heterozygous for two linked genes and reproducing by parthenogenesis, should be divisible, theoretically, into five groups in so far as the association of the two homologous chromosomes left in the egg is concerned:—complementary non-crossovers, identical non-crossovers, crossover and non-crossover, complementary crossovers, and identical crossovers. The absence of all groups involving crossovers except that of identical crossovers in Nabours' data would show that (1) crossing-over occurs either in the two strand stage or between both strands of one homologue and both of the other, and (2) the first maturation division is completely segregational. The grouse locusts, then, exhibit quite different conditions from those in *Drosophila* and *Habrobracon*, where crossing-over takes place only between two of the four strands and the first maturation division is at times segregational and at others equational. The alternative hypothesis to that of the suppression of the second maturation division is that both maturation divisions occur and the partheno-pro-

duced embryos start as haploid but later change to diploid as shown by Slifer and King for certain other grasshoppers. This would give an adequate explanation of Nabours' breeding experiments and of Robertson's cytological observations without forcing the adoption of the above two subsidiary hypotheses.

34. *Genetic Changes in Mice Induced by X-rays*: G. D. SNELL, University of Texas. An experiment to determine the effect of x-rays on the hereditary constitution of mice has shown that a high incidence of genetic change is induced. Alterations of the genetic constitution provisionally interpreted as translocations are present in more than one fourth of the F_1 mice. Males of the x-rayed generation came from one inbred stock (stock A), females from another inbred stock (stock B). Only males were rayed, the dose used ranging from 400 to 1200 r-units. To insure an efficient control, each stock A male was mated once before raying to produce an F_1 control litter and one or more times after raying to produce the F_1 test litter or litters. The F_1 individuals were back-crossed to stock B to furnish the BC generation. It was found that many of the BC litters in the rayed group were abnormally small, containing less than 5 young. The same F_1 individuals that threw small BC litters on the first mating usually threw them consistently if further matings were made. The result was a bimodal distribution of litter size, with one mode at 9 young per litter and another at 4 young per litter. Of 110 F_1 individuals in the x-rayed group, 29 produced litters that averaged 4 or less young. The control group was unimodal. Not one of the 98 F_1 control individuals produced litters that averaged less than 4.5 young. An embryological study has shown that the small BC litters are due to the degeneration of a part of the embryos, usually at or shortly after implantation.

35. *White Spotting in Peromyscus maniculatus*: R. R. HUESTIS and ELIZABETH BARTO, University of Oregon. Specimens of *Peromyscus* may exhibit a patch of white hairs in certain characteristic positions, viz.: (1) On the nose or forehead (star), ranging in size from a few white hairs to a patch running well back between the ears. Small stars are not uncommon in wild specimens. (2) On the normally black tail stripe, situated either terminally or subterminally, and extending from a few milli-

meters to several centimeters along the tail. (3) On the ventral surface. This patch of hairs, white to the base, may cover as much as half of the ventral surface, and is frequently associated with (2) above. It is less commonly found with star but appears with it more often than would be expected from chance association. Star behaves like a Mendelian recessive in all matings but those in which both parents are starred. In litters from these latter, young are obtained without stars, or with larger stars than those of their parents. Data upon the inheritance of a white tail stripe indicate that both dominant and recessive genes are involved. Ventral spot also has a complex type of inheritance. The common association of a ventral spot with a white tail stripe, or a star, may be due either to linkage or to a dual effect of certain of the involved genes. A new coat color, elsewhere reported as a yellow, but probably more comparable with brown agouti *Mus*, appeared as a segregant during this investigation.

36. *Linkage Groups in the Tomato*: J. W. MACARTHUR, University of Toronto. The tomato has 12 pairs of chromosomes, very equal in size and all relatively short, making great map lengths improbable. The number of genes definitely placed in groups or studied in all dihybrid combinations is 18, including three new ones: purple stem which turns green (a_2), wilted leaf (wt), and dwarf accentuator (d_2). The first 15 genes have already been placed in seven groups, and the last 3 appear from the tests to be independent of all these and of each other. There are thus 10 separate groups or markers, which may eventually be merged in part but are tentatively allocated to chromosomes as follows: I. $d-p-o-s$. II. r . III. $y-br$. IV. $c-sp$. V. $f-a-lf$. VI. l . VII. $h-u$. VIII. a_2 . IX. wt . X. d_2 .

37. *A Tri-generic Hybrid of Zea, Tripsacum, and Euchlaena*: P. C. MANGELSDORF, Texas Agricultural Experiment Station, and R. G. REEVES, A. and M. College of Texas. The hybrid of *Zea* × *Tripsacum* produces a small percentage of functional female gametes, all of which have the somatic number of chromosomes. This hybrid, when pollinated with *Euchlaena*, a third genus, exhibits a fertility of about six per cent. The triple hybrid resulting from this cross possesses 38 chromosomes comprising, presumably, one genom of 10 from *Zea*; one genom of 18 from

Tripsacum, and one genom of 10 from *Euchlaena*, thus combining in one plant all the germ plasm of three distinct genera. Various characters of each of the three parents are expressed in the hybrid, though, in general, it shows a greater resemblance to *Euchlaena* than to the other parents. Cytological studies at meiosis indicate that there is almost complete pairing of *Zea* and *Euchlaena* chromosomes to form 10 bivalents while the 18 *Tripsacum* chromosomes behave as univalents. There is little, if any, pairing of *Tripsacum* chromosomes with those of *Zea* or *Euchlaena*.

38. *Temperature, Crowding, and Quantity of Food as Factors in the Control of Sex in Moina macrocopa*: L. A. BROWN and A. M. BANTA, Transylvania University, Brown University, and Carnegie Institution of Washington. This exhibit in chart form sets forth the effects of these different factors upon male production with particular attention to differentiation between the effects of crowding and quantity of food. It shows that in control of sex by crowding quantity of food plays at most a very minor rôle in the result.

39. *Inheritance in Diploid Parthenogenesis Contrasted with Biparental Inheritance*: A. M. BANTA and THELMA R. WOOD, Brown University and Carnegie Institution. A general diagrammatic representation of results of studies with *Daphnia longispina*.

40. *Heat Induced Mutations in Drosophila and their Evolutionary Significance*. (15 min.) (Lantern): H. H. PLOUGH, Amherst College. A series of tests using the Goldschmidt-Jollos method of subjecting 6-day-old *Drosophila* larvae to sub-lethal temperatures confirms their report that an increased number of mutations appear in successive generations derived from the animals exposed. As reported last summer Plough and Ives found an increase in mutation rate of 3 to 5 times that in the controls, which is, however, much lower than that reported by Jollos. Since the number of flies examined total nearly 200,000, and most of the mutations have been located accurately, it is now possible to draw certain definite conclusions regarding the time of origin of these mutants and their evolutionary significance. A total of 17 visible mutations were found in 450 cul-

tures from offspring of individuals subjected to heat only once. Of these, 10 appeared in the first generation derived from parents each of which had been heated. Four of these were dominant or semi-dominant, and 6 were recessive. In order that recessives should appear in this generation the same mutation must have occurred in the germ cells of each parent. Thus the same gene change must have been induced in at least two individuals simultaneously. The remaining mutations appeared in successive generations including the eighth. Of these latter, 2 were clear-cut dominants. Since dominants appear in the generation derived from the germ cells in which the mutation occurs, it is clear that heat causes some effect (perhaps on the cytoplasm) which is carried over for many cell generations before a gene mutation takes place. The 17 mutations found include one from red to white eye, and one reverse mutation from Bar to normal. Only one (dominant glued eye) is a new mutation not already observed in control cultures. Except for an increase in mutation rate the results parallel those found for spontaneous mutations. The data indicate that high temperature has no effect on the direction of evolution, but because more mutants may be available to be acted on by selection, it may speed up the process. This is in accord with the calculations of Fisher based on *a priori* reasoning.

41. *A Contribution to the Theory of Dominance.* (15 min.): C. R. PLUNKETT, New York University. The following theory is based primarily on the work of the author and others on the developmental interaction of genetic and environmental factors: Most, or probably all, of the chemical and physical processes involved in development approach their end points asymptotically. In general, processes determined entirely by wild-type genes actually proceed to points very close to their asymptotes, while those affected by mutant genes are more usually terminated by extrinsic factors at points relatively far from their asymptotes. This results in a generally smaller variability of wild-type as compared with mutant characters and, incidentally, to a usual, though not necessarily universal, dominance of wild-type to mutant allelomorphs. This theory reconciles the apparently contradictory "physiological" and "evolutionary" theories of dominance and meets the chief objections to each of them. It explains dominance in terms of developmental processes, but

regards the dominance relation between any two allelomorphs as a function also of all other factors affecting development. It regards the usual dominance of wild-type genes as a result of selection, but, in general, merely an incidental result of selection in the direction of enhancement and stabilization of favorable characters. Only in cases of exceptionally high mutation rates would the existence of the heterozygous mutants in the population be an important contributory factor in this selection.

42. *The Effects of Temperature on the Mean Bristle Numbers of Scute 1 in Drosophila melanogaster.* (15 min.) (Lantern): GEORGE P. CHILD, New York University. The effects of temperature on the mean numbers of the various bristles affected by scute 1 were determined under carefully controlled genetic and environmental conditions. Curves obtained by plotting the mean numbers of each specific bristle against temperature showed a diversity of types: curves with a positive slope, with a negative slope, with a maximum and with a minimum. Preliminary studies indicate that in general the temperature effective period for any specific bristle covers the same definite portion of the time of development at all temperatures. When the period is determined by allowing the flies to develop first at a high temperature and then transferring them to a lower temperature, the temperature effective periods for all bristles occupy the same portion of the time of development. When the reciprocal experiment is performed, however, it is found that for certain bristles there is an additional temperature effective period prior to the one common to all bristles.

43. *The Effect of Temperature upon Eyeless and upon Eyeless-Bar Compounds in Drosophila melanogaster.* (15 min.) (Lantern): A. L. BARON, New York University. In an eyeless stock selected for uniformity of eye size, and showing no literally "eyeless" individuals, the facet number was found to be unaffected by temperature. No appreciable sex differences were observed. However in another eyeless stock which had been selected "down," i.e., for totally eyeless individuals, the percentage of eyes present increased with decrease of temperature, and a considerable sex difference was observed. Since the distribution of facets among the "eyed" flies of the low selected stock is not normal, i.e., practically all such flies have high facet

numbers, the effect of temperature in this stock can not be considered as influencing facet number, but rather some process operating on an all-or-none principle, thus changing the percentage of eyes but not necessarily the facet number. The combination of the uniform eyeless stock with Bar indicates that these genes do not operate independently, since the resulting facet number is smaller than would be expected on the basis of such independent action. Heterozygous eyeless has no detectable effect on Bar flies. The facet-temperature curve for BB ey ey individuals is very similar to the curve for homozygous double Bar, and that of the B+ ey ey individuals to that of homozygous Bar, the slope of the curve being determined, apparently, by the presence of the Bar gene and by the number of facets.

44. *Growth Rate, Metabolic Rate and Ultimate Body Size.* (10 min.): R. CUMMING ROBB, College of Medicine, Syracuse, N. Y. A correlation of my own endocrine growth studies pertaining to giant and pigmy rabbits with 500 unpublished basal metabolic observations on rabbits, graciously provided by Dr. David Marine, of New York City, has produced the following results: (1) Parallel to the fall of relative growth rate there is a decline of heat production—from 5 calories per kilogram per hour at the age of one month, to $2\frac{1}{2}$ cal/kg/hr at ten months. (2) The heat production of maturity (after termination of growth!) is the true maintenance level, and is characteristic of the race. Here it is about 60 cal/kg/day. (3) The daily amount of growth per gram of tissue is directly proportional to the daily heat production per gram in excess of the maintenance level. About 2 calories are expended with the production of each gram of new tissue. (4) the ultimate size tends to be inversely proportional to the maintenance level. Apparently mammals with a low heat maintenance requirement attain large size, whereas those with high requirements always remain small. (5) In general, the logarithm of ultimate body weight approximates an inverse linear function of the maintenance heat requirement per kilogram per day.

45. *Duration versus Rate of Growth in the Attainment of Large Size.* (5 min.): R. CUMMING ROBB, College of Medicine, Syracuse, N. Y. It is a familiar observation that large animals

and plants generally require a longer growth period than many of the smaller varieties. In the rabbit the instantaneous relative growth rate ($\frac{dW}{dt}$) in giant, pigmy and hybrid *males* is of the same order of magnitude at birth and for perhaps three months thereafter. Gradually their rates diverge, being more rapidly reduced in the smaller animals. The giants require some months longer to complete their growth. This phenomenon can be interpreted as the effect, *not* of differences in initial endowment, but of difference in negative acceleration acting upon the rate of synthesis (per gram of tissue) throughout postnatal life. (Metaphor: velocity and distance traveled by rough- and smooth-surfaced toboggans while descending same incline with same initial velocity; the latter goes further and loses speed more gradually.) An alternative interpretation has been based on observations upon the female. Admitting that the *smoothed averages* do present an abstract picture of a "consistently higher" relative rate (in that sex only) one must not forget that this is not necessarily true of individuals in that group, many giants falling below many of the pigmies. We may conclude that ultimate size is an equilibrium state characteristic of the organism. Its attainment is independent of either velocity or relative rate of growth at any instant (although these may show positive correlation with size.) The cannon-ball trajectory theory postulating simply "initial velocity" differences does not apply to the relative growth curve, and can not apply to the velocity curve of rabbit body growth.

SHORTER ARTICLES AND DISCUSSION

EFFECTS OF X-RADIATION ON PRODUCTION OF MOSAIC MALES AND ON SEX RATIO IN HABROBRACON¹

DURING the genetic work on the parasitic wasp, *Habrobracon juglandis* (Ashmead), there have been described (Whiting, P. W., '28; Whiting, P. W., and Anna R. Whiting, '27) a number of mosaic males obtained from heterozygous females. Up to the time of this experiment, however, only eighty-four such males had been counted and examined.

P. W. Whiting ('22) advanced the hypothesis that mosaic males in *Habrobracon* result from incomplete maturation of the egg. The hypothesis assumes that these irregular types come from unfertilized eggs of heterozygous mothers in cases where there was post-reduction and failure to extrude the second polar body, two oötidis with different genes taking part in development. Anna R. Whiting and Carey H. Bostian ('31) obtained further evidence substantiating this hypothesis. They state (p. 679), "Eye and wing mosaic males appeared only among progeny of heterozygous females since they are due to binuclearity of egg."

Goldschmidt and Katsuki (Goldschmidt, '31) in their work on *Bombyx mori* L., obtained results of interest in this connection. They found that gynandromorphs and somatic mosaics resulted from egg binuclearity and that the tendency to produce binucleate eggs had a certain definite Mendelian basis. They observed that certain strains of *Bombyx* produced high ratios of mosaics. By genetic tests they showed that the hereditary basis of mosaicism in these strains is a single recessive gene. By cytological analysis they showed that a high percentage of the eggs of individuals of this strain were binucleate.

While making certain linkage tests in *Habrobracon* the author found that crosses involving shot vein, sv, (wings) stock females

¹ The author wishes to acknowledge his sincere indebtedness to Professor P. W. Whiting, under whose supervision this work was done and to express his gratitude to Professor R. T. Hance, who, as head of the department of zoology, provided space and laboratory equipment. The Victoreen Dosimeter and much essential technical assistance has been provided by a grant to Dr. Whiting from the Committee on Effects of Radiation on Living Organisms (National Research Council).

(Whiting, P. W., '32) yielded an unusually large number of mosaic males (Table I). In cross A, shot vein females by ivory,

TABLE I
MOSAIC MALES APPEARING IN LINKAGE TESTS

Crosses		F ₁ females set.	F ₁ males		
			Regular	Mosaic	Per cent. mosaic
A	sv ♀ × o ¹ .ct ♂	38	416	7	1.66
B	sv ♀ × cl ♂	22	643	3	.46
C	sv ♀ × o.w ♂	20	433	0	0
D	sv ♀ × br ♂	18	506	0	0

o¹, (eyes), cut. ct, (wings) males, there were 423 F₂ males of which seven were mosaics. Of the 646 F₂ males obtained in cross B, shot vein females by club, cl, (legs) males, three were mosaics. Although no mosaics were noted in crosses C and D, also involving shot vein females, the high ratios obtained in crosses A and B indicated that the shot vein stock might be valuable material for the study of ratios of mosaics. Therefore, this stock was used as the basis of the present experiment, the purpose of which was to determine whether mosaicism could be increased by means of x-radiation.

Shot vein females were mated to ivory stumpy, st, (legs) males. The shot vein stock was derived from Minnesota yellow, M_y, (base of antennae) stock during the summer of 1930 and has been kept as a pure line since that time. During the same summer stumpy arose as a mutation and ivory stumpy males were derived. This combination has since been maintained by breeding to wild type (stock one) females.

The F₁ heterozygous females from the above cross (shot vein by ivory stumpy) eclosed in the vials and, consequently, some of them mated with their brothers. These matings were permitted with the expectation that gynandromorphs would appear. Approximately two-thirds of the F₁ females from each vial were placed in gelatine capsules and treated. The remaining one-third were used as controls. A Coolidge tube with a tungsten target was used. The conditions of the treatment were as follows: crest—76 K. V.; milliamperes—8; distance from target—15 centimeters; filter— $\frac{1}{2}$ millimeter aluminum; duration—8 to

10½ minutes. The dosage amounted to 2,000 Roentgen units, checked with a Victoreen dosimeter before and after each exposure.

MOSAICISM

Table II shows progeny produced by the 357 treated and 194 control females. As regards ivory and stumpy, mosaicism is readily determined. Individuals having only wing differences were not included since the factor shot vein has a variable somatic effect (Whiting, P. W., '32) sometimes affecting only one wing.

TABLE II
MOSAIC AND MUTANT MALES FROM X-RAYED AND CONTROL ADULT FEMALES,
FROM SV FEMALES × Ost MALES

F ₁ females set.	F ₁ progeny					
	Females	Gynandro- morphs	males			
			Regular	Mosaic	Per cent. mosaic	Mutations
357 x-rayed	1449	0	6176	18	.2914± .0462	0
194 control	2789	1	4041	3	.0742± .0289	3

From the experimental data certain interesting ratios were obtained. The percentage of mosaics among male offspring from treated females was $.291 \pm .046$; in the controls it was $.074 \pm .029$. The difference, $.217 \pm .055$ per cent, is more than three times the probable error. Therefore, we may assume that this x-ray dosage has significantly increased the rate of production of mosaic males.

The eighteen mosaic males from treated females included four involving ivory; two, stumpy; two, ivory and shot vein; three, stumpy and shot vein; and seven, ivory, stumpy and shot vein. The three mosaic males from control females included two involving ivory and one involving ivory, stumpy and shot vein.

It has been shown (Patterson, '31) that x-radiation increases mosaicism (gynandromorphism) in *Drosophila* in which case the basis for the phenomenon is chromosome elimination. The data presented in this paper demonstrate that mosaicism based on egg binuclearity is likewise increased by radiation.

Only one mosaic appeared from fertilized eggs, a gynandromorph of wild type traits, female anteriorly and male posteriorly. Since criteria for distinguishing gynandromorphs are different from those used for mosaic males data from fertilized eggs (females) are not comparable with those from unfertilized. On the basis of the ratio of mosaics among the male offspring of treated it might be expected that there would be a comparable ratio of gynandromorphs among the 1449 females. This would amount to 4.2 individuals. Since polyspermy, as well as egg binuclearity, probably occurs it is likely that some binucleate eggs develop into females on account of fertilization of both nuclei. Two gynandromorphs with patroclinous male parts have been thus interpreted (Whiting, P. W., '31).

MUTATIONS

Mutations rarely appear among sons of treated females, probably on account of simultaneous lethal effects on the zygote. It may be noted that the three mutations occurring in this experiment were among male progeny of control females (Table II).

Two of these appeared as single male mutants; one to fused, f, (antennae, tarsi, wings) the other to orange, o, (eyes) (tested by breeding). Mutations to fused have been previously noted five times. The complex of characters is unmistakable. Orange appears not infrequently among offspring of females heterozygous for ivory, its allelomorph. These two loci are then relatively unstable.

The third mutation is new; strap, sr, reduces venation and narrows wings. Strap males appeared among regular brothers in the third, fifth and sixth vials from one female. The mutation probably occurred in an oögonial cell.

SEX RATIO

It has been previously noted (Whiting, P. W., '29, p. 275; Bostian, Neita C., '31) that the percentage of female offspring from x-radiated mothers, mated previous to treatment, has been markedly decreased. To observe the bearing of the present data on this point, a summary of those fraternities containing both male and female offspring was made (Table III). The difference in percentage of females, $20.8 \pm .007$, is very significant and in favor of the controls in spite of the fact that the omission of unisexual fraternities would tend to increase the ratio of females among the treated by eliminating extreme cases.

TABLE III
SEX RATIO IN BISEXUAL FRATERNITIES

F ₁ females set	F ₂ progeny					
	Males	Gynandro- morphs	Females	Per cent. females	Sons per mother	Total per mother
182 x-rayed	2669	0	1449	35.2 ± .502	14.66+	22.63-
138 control	2191	1	2789	56.0 ± .474	15.88-	36.09+

It might be supposed that fertilizability of eggs was reduced by the treatment. This possibility is precluded as shown by Neita C. Bostian ('31) who obtained normal sex ratio from treated virgin females subsequently mated to untreated males. Since it is known that x-radiation injures sperm in the males it may be supposed that sperm in seminal receptacles are likewise affected.

Fecundity of females is greatly reduced by extreme treatment, but with this relatively light dosage there is little effect on unfertilized eggs (sons per mother, Table III). Total progeny per mother is, however, greatly reduced, the striking deficiency of females not being compensated by an increase in males. Such an increase would be expected were it not for the fact that treatment of eggs cuts down the productivity in general. The difference between reduction in sons per mother, 7.62 per cent. and reduction in total offspring per mother, 37.32 per cent. is 29.70 per cent. It may be suggested that sperm affected by x-radiation are, in part at least, able to fertilize eggs and that such fertilization is lethal.

The presence of dominant lethals induced in sperm of *Drosophila* has been postulated by Muller ('27) to account for partial sterility of x-rayed males. Female ratio was reduced on account of dominant lethals in the x-chromosome. The work was checked by egg counts. In *Habrobracon* we may expect female ratio to be much more reduced than in *Drosophila* since impaternate males will be unaffected.

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THE PHASES OF AFRICAN LOCUSTS¹

WHEN I visited Pretoria last year I was very much impressed by the amount and character of the entomological work done at the university, the Transvaal Museum, and also at the station for veterinary research at Onderstepoort. In the field of pure taxonomy, nothing could exceed the beauty and thoroughness of the work on Pyraloid moths by Professor A. J. T. Janse. The researches on the genetics of honey-bees, the natural history of termites, the investigations concerning ticks and mosquitoes, the orderly arrangement of the great collections in the museum, all combined to make one think of Pretoria as a desirable place to live and work. But most wonderful of all, perhaps, was the work of Professor Faure on the phases of African locusts. He was good enough to demonstrate it to us in detail, and now we have the publication of his results, "made possible by the generous assistance of the Carnegie Corporation of New York, received

¹ "The Phases of Locusts in South Africa," Jacobus C. Faure, *Bulletin of Entomological Research*, September, 1932. 112 pp. and 25 plates.

through the Research Grant Board of the Union of South Africa." Certainly, in supporting these experiments and their publication, the Carnegie Corporation made excellent use of its funds, though one can not help regretting that the Union of South Africa did not feel able to assume this burden—a very light one for so great a country, especially in view of the economic interests involved. However, considering some of the recent "economies" in Washington, the United States certainly can not point the finger of reproach.

Faure's work is a good type of what we like to call the new entomology, or shall we say synthetic entomology? It combines the methods of taxonomy, ecology, and physiology, of observation and experiment, all converging to make an intensive study of the problem in hand. But by virtue of these good qualities, it simultaneously gains positive results, and opens up a multitude of problems for future investigation.

The occasion for these researches grew out of the remarkable theories of Uvarov, the learned Russian entomologist who now has charge of the Orthoptera in the British Museum. To the perennial and insistent question, "Why do locusts swarm?," Uvarov answered by saying that the migratory species were so constituted that when the population became very dense, the insects actually changed their characters, both appearance and behavior. The coloration was affected, with the development of a pattern of black and orange, and the wings were longer. Thus these insects were dimorphic or polymorphic, presenting phases which owed their development to the conditions of life. These were called "phase solitaria," the non-swarmling types, and "phase gregaria," which formed the migratory swarms. The term "transiens" was applied to the intermediate forms.

Such an extraordinary theory naturally led to much discussion, but it was supported by many facts, gleaned from various parts of the world. Since locust swarms do so much damage in Africa, Professor Faure decided to carry on extensive experiments, to determine the precise facts in relation to the important South African species. The principal one is *Locustana pardalina* (Walker), and the others are *Nomadacris septemfasciata* (Serville), *Locusta migratoria migratorioides* Reiche and Fairmaire, and *Schistocerca gregaria* (Forsk.). The Uvarov theory was confirmed in every particular, and all the details of the work

are explained, with colored plates drawn by Professor Janse. It is well known that in some Orthoptera the various color phases are hereditary, and subject to Mendelian laws. The coloration of the gregaria phase is only hereditary in the sense that the insects possess a mechanism which permits—indeed compels—the appearance of the special colors under special circumstances. These circumstances are essentially those connected with excessive crowding, which leads to almost perpetual activity. “This activity leads to a greatly increased rate of metabolism: There must be nervous activity to produce the muscular work; the muscles need more food, circulation of the blood must be increased, and so on. Meanwhile, the black and orange coloration develops, and this doubtless causes a rise in the body temperature, as compared with that of a green or gray hopper, due to the greater absorption of heat by the pitch black cuticula. Possibly this high rate of metabolism has certain features in common with the condition described as fever in a vertebrate. As the body temperature rises, the moisture requirements increase, leading to the voracious appetite for all manner of moist substances that is characteristic of the hoppers of the phase *gregaria*. The gregariousness leads the organism into a kind of vicious circle from which it can not escape; activity leading to black coloration, which causes a rise in body temperature, and this in turn producing still greater activity.” (p. 365.) Professor Faure proposes the term *locustine* “for the products of this excessive metabolism which lead to the development of the *gregaria* coloration.” This is merely a temporary convenience, the name does not apply to anything which can be defined in chemical terms. On mating *gregaria* with *gregaria*, both *gregaria* and *solitaria* were produced, according to the condition of life of the young hoppers. This is very strikingly shown by a photographic illustration. There seemed to be inheritance of acquired characters, when the first stage larva from *gregaria* would show the dark coloration. But this was due to the “*locustine*” in the egg, and disappeared in the second stage if the insect was reared alone.

It will be readily appreciated that these observations are equally important from the standpoint of general physiology and genetics, and that of economic entomology. It will now be possible to predict locust swarms while the insects are still in the immature stage, and take measures to “nip them in the bud.”

It is now possible to understand why the famous Rocky Mountain locust, *Melanoplus spretus*, has vanished off the face of the earth. Formerly it appeared in vast and destructive swarms; now we can not find a single individual. On taxonomic grounds, Hebard some years ago decided that it was only a race or phase of *Melanoplus mexicanus*, more commonly called *M. atlantis*, an abundant resident, non-migratory species. It differs principally by the much longer wings. No doubt *spretus* was the phase *gregaria* of *mexicanus*, and I understand that experiments are now being carried on in Minnesota, to see if it is possible to produce *spretus* from *mexicanus*, using the method of Faure.

An albino phase of *Locustana pardalina* was found, and this was incapable of taking on the *gregaria* coloration, even when crowded. It proved on breeding to be a mendelian recessive.

Other experiments were made, showing that the coloration of the hoppers was also affected by the color of their surroundings. Individuals raised in boxes painted different colors showed an extraordinary power of taking on the color of the box, black, orange or grey. The color green seemed to follow different laws, as is discussed at length. Certain non-migratory locusts, of the genera *Oedaleus* and *Acanthacris*, though very variable in color, could not be stimulated by crowding to develop any of the "*gregaria*" characters.

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GEOGRAPHICAL VARIATION IN LADY-BEETLES

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THE INTRASPECIFIC AND THE INTERSPECIFIC VARIABILITY

THE coleopterous family Coccinellidae presents very favorable material for studies on variation. The color-pattern of the elytra and of the pronotum is very widely variable in many species. The variations may be arranged in series beginning with entirely light (yellow or red) elytra, ranging through light elytra with a number of dark (black or brown) spots, dark elytra with light spots, and ending with dark, unspotted elytra. The intraspecific variability is not infrequently discontinuous, the intergrades between the different patterns being rare or absent.

Large groups of related species and genera exhibit parallel series of patterns, upholding the rule of homologous series in variation, formulated by Vavilov (1922). Thus, only a few of the more than one hundred patterns that are known to occur in the species of the genera *Coccinella*, *Semiadalia*, *Adonia* and *Hippodamia* are restricted to a single species. A large majority of these patterns reappear in several or in many species. The similarity of the homologous patterns in different species is often striking (compare Figs. 2 E and 3 H, Figs. 2 L, 4 D and 4 G, Figs. 2 I, 4 J and 5 D). Homologous varieties of different species may appear even more similar to each other than do different varieties of the same species. However, the different varieties of the same species, at

least those found in the same locality, intercross freely in nature as well as in experiment, while different species are, as far as known, intersterile (Lus, 1928).

THE GEOGRAPHICAL ELEMENT IN VARIATION

The different patterns known in a given species do not occur equally frequently in every part of the area inhabited by the species. In some sections of the specific area a majority of the population may consist of individuals having a pattern or patterns which are rare or absent in other sections of the same area. The species becomes, thus, differentiated into geographical races (subspecies). Each of the subspecies is characterized

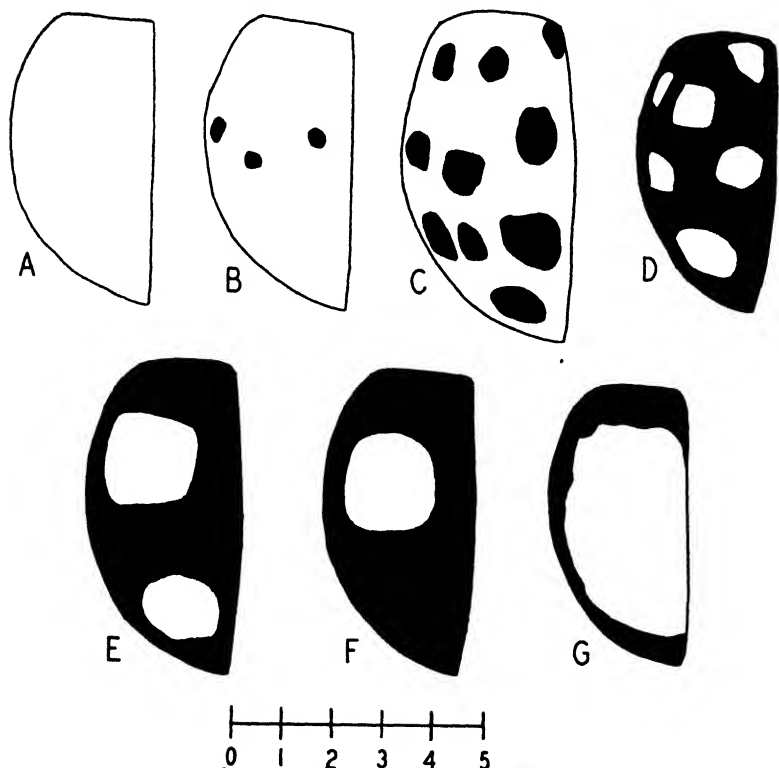


FIG. 1. *Harmonia axyridis* Pall. A—*var. succinea* Hope; B—*var. frigida* Muls.; C—*var. novemdecimsignata* Fald.; D—*var. axyridis* Pall.; E—*var. spectabilis* Fald.; F—*var. conspicua* Muls.; G—*var. aulica* Muls. The scale in this and in the following figures represents five millimeters.

by a definite frequency of the different patterns in the population. This situation may be illustrated by the example of the Asiatic species, *Harmonia axyridis* Pall.

Harmonia axyridis Pall. occurs in the southern stretch of Siberia, from the Altai Mountains to the Pacific, in Manchuria, China and in Japan. The western part of this area, extending from Altai to Lake Baikal, is inhabited by var. *axyridis* (Fig. 1 D, Table 1). Individuals having the pattern of var. *19-signata* (Fig. 1 C) occur in this region only as an exception. East from Lake Baikal var. *axyridis* loses rapidly its predominance, it is only seldom found in the Maritime Province and in Japan, and is not known to occur in China (Table 1). The variety *19-signata* is the most frequent one in the eastern part of the specific area; var. *spectabilis* (Fig.

TABLE 1

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Harmonia axyridis*
(IN PER CENT.)

	<i>succinea</i>	<i>frigida</i>	<i>19-signata</i>	<i>axyridis</i>	<i>spectabilis</i>	<i>conspicua</i>	<i>aulica</i>	n
Altai Mts.			0.05	99.95				4013
Yeniseisk Province		0.9		99.1				116
Irkutsk Province			15.1	84.9				73
Transbaikalia (western part)		4.9	45.9	49.2				61
Amur Province	7.3	29.3	41.5					41
Maritime Province (Khabarovsk)	18.6	18.1	38.7	0.2	13.4	10.7	0.3	597
Maritime Province (Vladivostok)	16.9	31.1	37.6	0.8	6.0	6.8	0.8	765
Manchuria	12.9	32.8	34.0		11.2	8.6	0.5	232
Korea	28.1	26.6	26.6		6.2	12.5		64
Japan		16.4	3.0	4.5	16.4	59.7		67
China (Chi-Li, Shan-Si, Shan-Tung)	36.8	12.5	27.0		12.5	10.5	0.7	152
China (Kan-Su, Sze-Chuan)	3.7	26.0	40.7		11.1	14.8	3.7	54

The column marked "n" in this and in the following tables indicates the number of individuals studied from a given region.

1 E) and var. *conspicua* (Fig. 1 F) are very frequent in Japan, less so in eastern Siberia and in China, and do not occur at all west of Lake Baikal.

The species *Harmonia axyridis* is, thus, segregated into several geographical races. The western race is well differentiated from others, since the population of the western race consists almost exclusively of individuals having the pattern *axyridis* (Table 1), and this pattern occurs in other races only as an exception. The eastern-Siberian, the Japanese and the Chinese races differ from each other only in the average frequencies of the occurrence of the different patterns in the population.¹

The degree of differentiation of the geographical races may vary greatly. In some species the geographical races are merely indicated, the relative frequency of the various patterns being only slightly different in different parts of the specific area. Other species are subdivided into more or less sharply defined races. In extreme cases nearly 100 per cent. of the population of each race may exhibit a pattern or patterns which are not found at all in individuals belonging to other races. The different degrees of differentiation of the geographical races may be interpreted as different stages of the same process. What we observe in various species at our time level, are the young or slowly differentiating races on one hand, and the old, well-formed races, on the other. A comparative study of the different species may give us an insight into the process of the evolutionary divergence in time and in space.

DIFFERENT STAGES OF THE DIFFERENTIATION OF THE GEOGRAPHICAL RACES

Adonia variegata Goeze inhabits almost the whole Palaearctic region, and, in addition, the eastern part of

¹ The variability of *Harmonia axyridis* is discussed in more detail in Dobzhansky, 1924. It is worth mentioning here that the varieties of this species differ from each other only or mainly in coloration, and do not differ in the structure of the genitalia.

Africa. The color pattern of this species is very variable. The typical pattern (Fig. 2 E) consists of seven black spots on each elytron. The decrease or the increase of pigmentation leads to the disappearance or to fusion of some or of all of these spots. More than eighty different patterns occur in the species; some of them are represented in Fig. 2. The geographical distribution of the patterns is shown in Table 2

TABLE 2

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Adonia variegata* (IN PER CENT.)

	<i>immaculata</i>	<i>constellata</i>	<i>carpini</i>	<i>litigiosa</i>	Some spots absent	<i>variegata</i> <i>typica</i>	Confluent spots	n
Kiev	0.2	39.2	23.3	9.8	9.4	13.2	4.9	1590
Crimea	0.3	33.7	17.7	11.9	13.3	19.6	3.6	362
Uralsk, Turgai and Ak- molinsk Provinces		28.6	14.3	16.8	13.4	21.8	5.1	119
Transcaucasia	0.5	40.1	26.4	11.6	4.2	11.1	6.4	189
Persia	2.0	53.1	14.3	7.1	14.3	8.2	1.0	98
Transcaspia	2.2	32.6	19.6	10.9	7.6	20.6	6.5	92
Semirechensk Prov.	0.3	27.1	17.2	13.0	11.0	24.8	6.5	354
Pamir and Darwaz		1.5	6.0		0.8	41.0	50.7	134
Chinese Turkestan		4.5	6.4	14.8	3.5	42.6	28.2	202
Ordos and Ala-Shan		2.7	21.9	4.1	1.4	27.4	42.5	73
Northern Mongolia		15.1	19.1	8.6	1.3	30.2	25.6	152
Transbaikalia		5.8	17.4	8.2		46.5	22.1	86
Maritime Province			18.2			50.0	31.8	22
Yakutsk Province						18.3	81.7	115
Abyssinia						3.6	96.4	56

(patterns involving simultaneous disappearance of some of the spots of the typical pattern and fusion of others are included under the heading "confluent spots"). An area extending from Europe to Caucasus, Persia and the plains of Turkestan is inhabited by a lightly pigmented race. In this region varieties *immaculata*, *constellata*, *carpini*, *litigiosa*, and other varieties having some spots missing clearly predominate. Persia seems

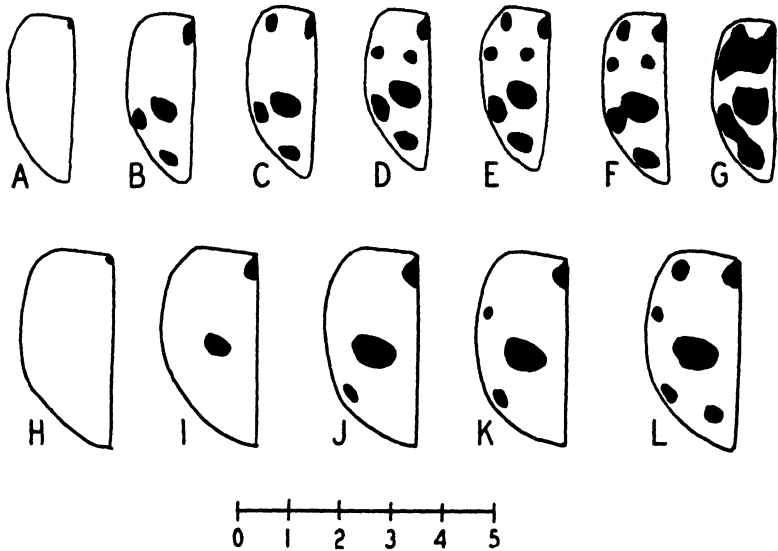


FIG. 2. A-G—*Adonia variegata* Goeze; H-L—*Coccinella quinque-punctata* L.; A—var. *immaculata* Gmel.; B—var. *constellata* Laich.; C—var. *carpini* Geoffr.; D—var. *litigiosa* Wse.; E—the type form; F and G—some of the varieties having confluent spots; H—var. *minckwitzii* Hänel.; I—var. *rossii* Wse.; J—the type form; K—var. *simulatrix* Wse.; L—var. *arthurica* Jacobson.

to be inhabited by an especially light race. Eastward from this area, starting from Pamir and the high mountainous plateaus of eastern Bokhara (Shughnan, Roshan, Darwaz) and up to the northeastern part of Siberia (Yakutsk), there is found a heavily pigmented race in which the var. *typica* and varieties having confluent spots predominate. The process of the accumulation of pigment reaches its peak in Yakutsk Province. Another very dark race is found in the part of the area inhabited by *Adonia variegata* which is furthest removed from Yakutsk Province, namely in Abyssinia and in east-central Africa (Lake Tanganyika).

In spite of the considerable difference between the frequencies of the various patterns in the different regions, *Adonia variegata* may be taken as an example of a species in which the geographical races are merely indicated. Indeed, in no region are one or a few patterns not found

elsewhere established to the exclusion of all other patterns. The typical pattern of the species is nowhere very rare, and certain patterns involving a confluence of spots are also found practically in the entire area inhabited by the species. *Hippodamia convergens* Guer. may be referred to as a species which is still less differentiated geographically than *Adonia variegata*. *Hippodamia convergens* is common almost everywhere in North America. The typical pattern of the species, which is very similar to the typical pattern of *Adonia variegata* (Fig. 2 E), is by far the most frequent one in any part of the specific area. However, in the population living in California from 2 per cent. to 10 per cent. of individuals have some of the spots missing. In the eastern states less than 1 per cent. of the population have some of the spots missing.²

Anatis ocellata L. is more differentiated geographically than either *Adonia variegata* or *Hippodamia convergens*. The species has twenty (typica) or eighteen (var. *linnaei*) black spots on each elytron. These spots may disappear or may fuse with each other into longitudinal stripes (Fig. 5 E, F, and G). In Europe individuals with all spots fused into stripes (Fig. 5 G) apparently do not appear, but individuals with spotless elytra are frequent. In Siberia the fusion of spots becomes more and more frequent as one proceeds eastward, and east and northeast of Lake Baikal individuals with striped elytra predominate (Table 3). In Maritime Province the population is, however, again less dark on the average.

Coccinella quinquepunctata L. (Fig. 2 H-L) is represented in Europe by the so-called typical form (Fig. 2 J) having five black spots on both elytra (Table 4). The number of spots, as well as the size of the spots, increases as one proceeds northeastward. In Yakutsk Province practically the entire population consists of var. *arthur-*

² The frequency of the different patterns in *Hippodamia convergens* was studied by Johnson (1910). His data are misleading, since he included under the name "convergens" at least three different species.

TABLE 3

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Anatis ocellata* (IN PER CENT.)

	No spots (bicolor)	Some spots absent	typica and linnaei	Spots confluent (hebraea)	n
Ukrainia	26.6	20.0	53.4		15
Vyatka Province	3.4	20.7	75.1	0.8	1009
Altai Mts.	3.1	7.8	64.1	25.0	64
Yeniseisk Province	1.4	10.0	71.4	17.2	70
Irkutsk Province	2.5	6.8	60.1	30.5	118
Transbaikalia	1.8	3.6	34.6	60.0	55
Amur Province			35.0	65.0	20
Maritime Province	8.3	8.3	50.0	33.3	24
Yakutsk Province			51.1	48.9	47

ica, having eleven large spots. Conversely, the Caucasian race is lighter than the European race: Caucasian individuals have only three spots or no spots (Table 4). Recently *Coccinella quinquepunctata* was found in the northeastern part of Turkestan (in Sungarian Ala-Tau). The few specimens known from there are all spotless.

TABLE 4

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Coccinella quinquepunctata* (IN PER CENT.)

	No spots (minckleyi)	3 spots (rossi)	5 spots (typica)	7 spots (stimulatrix)	9 spots	11 spots (arthurca)	n
Caucasus	4.7	95.3					64
Kiev		0.09	98.2	1.7	0.1		1100
Vyatka Province			97.9	2.1			325
Tomsk Province			7.1		51.8	41.1	56
Yeniseisk Province			36.6	9.7	19.5	34.2	41
Irkutsk Province						100.0	31
Transbaikalia					10.5	89.5	19
Yakutsk Province					1.1	98.9	88

This fact is interesting because the region lying between Caucasus and the northeastern Turkestan (Kirghiz Steppe) is inhabited by a race similar to that living in Europe. It is not unlikely that the similarity between the Caucasian and the Turkestanic races is a result of a process of parallel development.

Hippodamia tredecimpunctata L. (Fig. 3 G, H and I) inhabits nearly the entire Holarctic region. In Europe the spotted var. *typica* predominates (Table 5) but indi-

TABLE 5

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Hippodamia tredecimpunctata* (IN PER CENT.)

	No spots (<i>signata</i>)	3 to 11 spots	13 spots (<i>typica</i>)	Some spots confluent	n
Kiev	0.2	0.2	91.6	8.0	585
Askania-Nova	2.9		94.2	2.9	102
Astrakhan	3.0		97.0		33
Transcaucasia	87.7	4.1	8.2		73
Tashkent	99.3		0.7		153
Ferghana	100.0				111
Semirechensk Province			100.0		10
Ordos	16.7	5.6	77.7		18
Lower Amur			70.0	30.0	177
Yakutsk Province			85.5	14.5	55
Kamchatka			12.5	87.5	8

viduals having a few spots missing or confluent are also found. The entirely spotless var. *signata* is not known to occur in Europe, except a few specimens found in southern Russia (Kiev, Askania-Nova, Astrakhan, see Table 5). The situation is reversed in Transcaucasia, Persia and the southern part of Turkestan. In these countries the var. *typica* is not found at all, or is found only as an exception; it is replaced by the variety *signata*. In northern Turkestan and in Siberia the population consists of var. *typica* and of varieties having confluent spots. The frequency of the latter varieties consistently

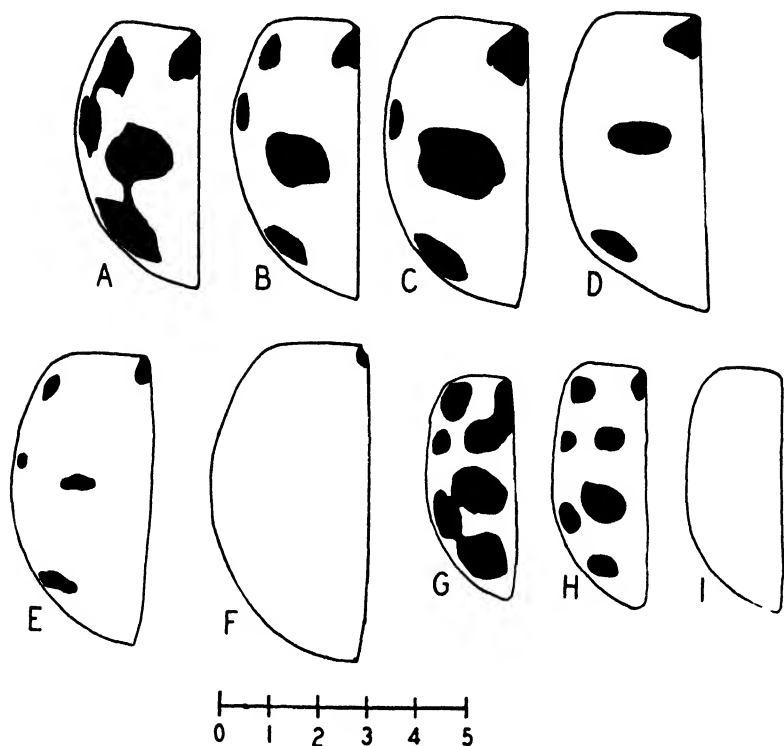


FIG. 3. A and B—*Coccinella novemnotata* Hbst.; E—*Coccinella novemnotata* Hbst. var. *degener* Csy.; F—*Coccinella novemnotata* Hbst. var. *franciscana* Muls. C—*Coccinella divaricata* Ol. var. *typica*; D—*Coccinella divaricata* Ol. var. *distincta* Fald. G—*Hippodamia tredecimpunctata* L. var. *contorta* Wse.; H—*Hippodamia tredecimpunctata* L. var. *typica*; I—*Hippodamia tredecimpunctata* L. var. *signata* Fald.

increases as one proceeds in the northeastern direction, and reaches its peak in Kamchatka. In Canada and United States the var. *typica*, similar to that found in Europe, is again met with.

In *Hippodamia tredecimpunctata* one may speak, consequently, of the existence of two subspecies. One of them, *signata*, occupies Transcaucasia, Iran and Turkestan, and the other, *typica*, inhabits the rest of the specific area. Within the region of each subspecies individuals having the color-pattern of the other subspecies are practically absent. There exists, however, a broad intermediate zone in which *signata* and *typica* occur together.

The intermediate zone includes the southern part of Russia, a part of China (Ordos) and, perhaps, Transcaucasia. A step further from the condition found in *Hippodamia tredecimpunctata* leads to the formation of two races entirely restricted to their respective geographical regions. This condition is approached by *Synharmonia conglobata* L. The European race of this species has pinkish elytra with eighteen black spots, which may be separate or fused with each other. The frequency of individuals with fused spots is higher than 50 per cent. in the European race, and only 3.3 per cent. in Transcaucasia (the latter figure is based on 245 specimens from Erivan, Transcaucasia). In the southern part of Turkestan an entirely different race (var. *buphthalmus*) is found. Elytra are yellowish with eighteen light-brown spots, each having a yellow area in the center. More than 200 specimens coming from various localities in Turkestan (mainly the environs of Tashkent and Fergana) were studied, and all showed the characteristics of var. *buphthalmus*. Chinese Turkestan, Songaria, and the deserts lying between Mongolia and Tibet are also inhabited by var. *buphthalmus* (Kashgar, Kilian, Sanju, Khotan, Keria, Kurla, Aksu, Manas, Barkul, Khami, Sadjou, western Kan-su, central Mongolia, 524 specimens of var. *buphthalmus* and no specimen of var. *typica* examined). The only region in which var. *typica* and var. *buphthalmus* occur simultaneously is the Semirechensk Province (northeastern Turkestan). In the southern part of this province (south of the river Ili, mainly environs of Verny) 89 per cent. of specimens found belong to var. *buphthalmus* and 11 per cent. of the black-spotted varieties (54 specimens examined). In the northern part of the same province (environs of Lepinsk) only 26 per cent. of individuals are var. *buphthalmus* (23 specimens examined). In Siberia the species under consideration is rare, but all the specimens known from there have black spots.

THE "CENTRA" OF LIGHT AND OF DARK FORMS

As stated above, there is observed a parallelism in the variability of the related species and genera. Homologous varieties of different species may be more similar to each other in appearance than the different varieties of the same species. This parallelism is, probably, due to the essential similarity of the germ-plasms of the related species. This is the genotypical parallelism. A thorough discussion of the genotypical parallelism lies beyond the scope of the present article. A different kind of parallelism is observed if one takes into account the geographical distribution of the various color-patterns. One may call it the geographical parallelism. It manifests itself in that the various species inhabiting a given region usually have more similar patterns than each of these species has in regions widely distant from each other. In some regions most species are represented by light, scarcely pigmented varieties. Such regions may be called centers of light forms. In other regions most species show an extensive development of dark pigmentation. These regions are centers of dark forms.

Two centers of light forms are apparent in the Northern Hemisphere. One of them lies on the Eurasiatic continent, in the southern part of Turkestan and in Persia. The second is found in North America, in California, Arizona, and, probably, in northern Mexico. The center of the dark forms lies in northeastern Asia (Yakutsk Province, Kamchatka). As shown above, the populations of *Adonia variegata*, *Anatis ocellata*, *Coccinella quinquepunctata* and *Hippodamia tredecimpunctata* in which dark patterns are most abundant are found invariably in northeastern Siberia. Turkestan is inhabited by the least pigmented varieties of the species just mentioned (except *Anatis ocellata*, which does not occur in Turkestan) and of *Synharmonia conglobata*.

Species inhabiting both Eurasia and North America are most important from the standpoint of the problem

of the centers of light and dark forms. One such species is *Coccinella transversoguttata* Fald. The race of this species inhabiting Siberia and Mongolia is the most extensively pigmented. Within the confines of this race the so-called typical form (Fig. 4 A) is decidedly predominant (Table 6). Alaska, Canada, the northern,

TABLE 6

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Coccinella transversoguttata* (IN PER CENT.)

	Darker than <i>typica</i>	<i>typica</i>	Bands partly broken into separate spots	11 separate spots	0 to 9 sepa- rate spots	n
Tomsk Province		100.0				102
Yenisseisk Province		100.0				17
Irkutsk Province	1.5	97.0	1.5			65
Transbaikalia	3.1	93.8	3.1			96
Amur Province	2.0	93.9	4.1			46
Tannu-Tuva (Urjanch Prov.)		94.7	5.3			38
Northern Mongolia	0.6	91.8	7.0	0.6		172
Central China (Ala-Shan, Kan-Su Provinces)		33.4	13.0	50.7	2.9	69
Sungaria		8.1	6.1	78.7	7.1	99
Semirechensk Province				89.0	11.0	45
Chinese Turkestan				92.6	7.4	190
Tibet				90.0	10.0	10

central and the eastern parts of the United States are occupied by a race the elytral pattern of which differs from that of var. *typica* by the absence of the spot lying near the external border of the elytron (Fig. 4 B). Individuals having the pattern represented in Fig. 4 B occur, however, also in the southern part of Siberia and in Mongolia. Likewise, the pattern shown in Fig. 4 A is not rare in Canada and in northern United States. In the western United States a progressive reduction of pigmentation is observed as one proceeds in the south-westerly direction. The transverse bands forming the pattern of var. *typica* disintegrate into separate spots;

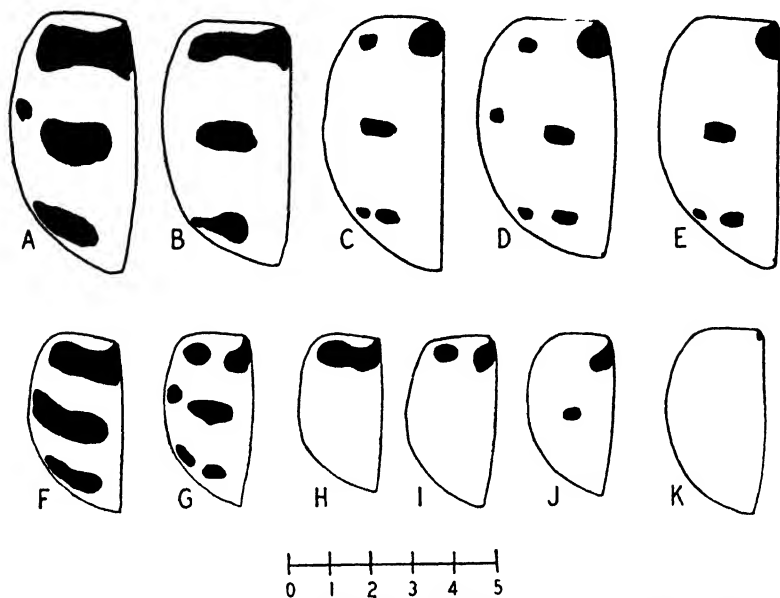


FIG. 4. A-E—various patterns of *Coccinella transversoguttata* Fald.; F-K—*Coccinella trifasciata* L.

the resulting pattern is shown in Fig. 4 C (var. *nugatoria*). Var. *nugatoria* is as frequent, or more frequent, than the typical form in Utah, New Mexico, Arizona and California. In Mexico var. *nugatoria* seems to be decidedly predominant.

The process of depigmentation takes place also in Asia as one proceeds from eastern Siberia in the southwesterly direction (Table 6). A pattern rather similar to that of var. *nugatoria* (eleven widely separated black spots, Fig. 4 D) is found in some specimens from Mongolia, and becomes the predominant pattern in central China, Sungaria and the adjacent mountain ranges. The depigmentation progresses still further in the Tyan-Shan mountains, Chinese Turkestan and in Tibet. Some individuals found in these countries have less than eleven black spots. Races living in Central Asia and in southwestern United States are, thus, more similar to each other than either of them is to the Siberian and the Canadian races.

Similar phenomena are observed in *Coccinella trifasciata* L. and *Coccinella nivicola* Men. (Fig. 4 F-K and Fig. 5 A-D, respectively). The typical form of *Coccinella trifasciata* (Fig. 4 F) constitutes nearly 100 per cent. of the population in Siberia, most of Canada (except British Columbia) and most of the United States (except the Pacific Coast). In the southern parts of Siberia and Mongolia the transverse bands forming the typical pattern become narrower, and show a tendency to disintegrate into separate spots. The same tendency finds a much stronger expression on the Pacific Coast of America, west of the Cascades and Sierra Nevada. In British Columbia, Washington and northern Oregon the patterns shown in Fig. 4 J and K predominate. In southern Oregon and northern California the pattern 4 G is the most frequent one. In the San Francisco region the patterns 4 H and I are the commonest.

Coccinella nivicola Men. is represented in Siberia by the variety having broad black bands (Fig. 5 A). In Canada and northern United States lives a race (var. *monticola*) having narrower bands (Fig. 5 B). A similar narrow-banded race is found, however, also along the southern boundary of the area occupied by the species under consideration in Asia (western Mongolia, Sungaria, northern Tyan-Shan). In America, between the Rocky Mountains and the Pacific Coast the depigmentation progresses still further. The bands become narrow and disintegrate into spots (var. *alutacea*, Fig. 5 C). In the deserts of Utah and Nevada the lightest race (Fig. 5 D) is found.

The behavior of the two closely related species, the American *Coccinella novemnotata* Herbst. and the Eurasiatic *Coccinella divaricata* Ol., is remarkable. In southern California (Los Angeles region) lives the unspotted *Coccinella novemnotata* var. *franciscana* (Fig. 3 F). In central California (San Joaquin valley), Arizona and New Mexico the unspotted form occurs together with individuals having from one to nine small black

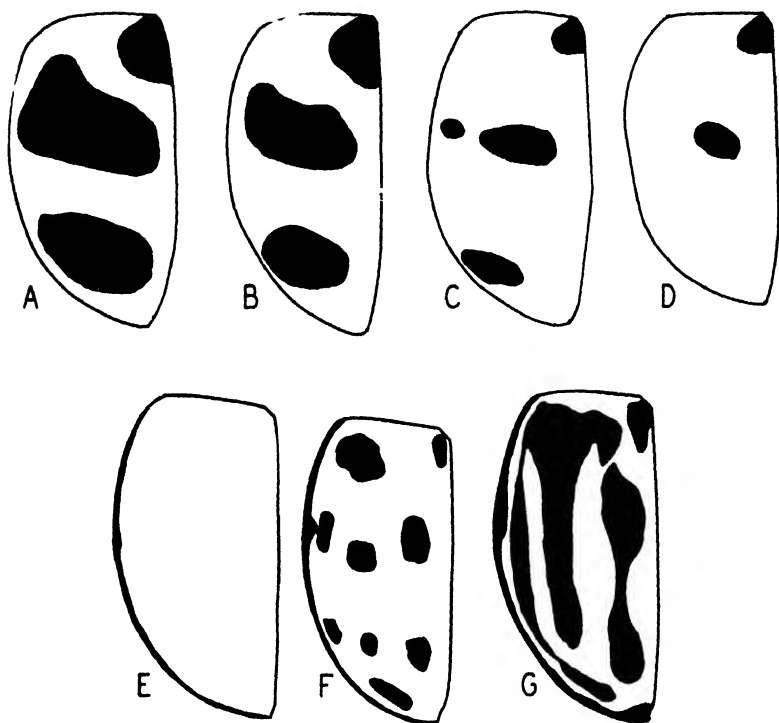


FIG. 5. A-D—*Coccinella nivicola* Men. A—*Coccinella nivicola* Men. type form; B—*Coccinella nivicola* Men. var. *monticola* Muls.; C and D—*Coccinella nivicola* Men. var. *alutacea* Csy.; E—*Anatis ocellata* L. var. *bicolor* Ws.; F—*Anatis ocellata* L. var. *typica*; G—*Anatis ocellata* L. var. *hebraea* L.

spots. Oregon, Washington, British Columbia and the states lying between the Sierra Nevada and Rocky Mountains are inhabited mainly by var. *degener* (Fig. 3 E, nine small spots). In Canada and in states lying east of the Rockies lives the typical form of the species (Fig. 3 B, nine large spots). In the eastern and southern states individuals with some of the spots fused are rather common (Fig. 3 A).

In eastern Siberia a variety of *Coccinella divaricata* is found (Table 7) which is similar in appearance to the typical form of *Coccinella novemnotata* (Fig. 3 B). In Transbaikalia about 20 per cent. of individuals have some of the spots fused together. West and southwest of

TABLE 7

RELATIVE FREQUENCY OF THE DIFFERENT PATTERNS IN *Coccinella divaricata*
(IN PER CENT.)

	5 spots (<i>distincta</i>)	7 spots (<i>typica</i>)	9 spots (<i>dominica</i>)	Some of the spots confluent (<i>interfusa</i>)	n
Kiev		91.9	8.1		344
Transcaucasia	90.0	10.0			10
Somirechensk Province		100.0			35
Tomsk Province		93.6	6.4		47
Irkutsk Province		56.1	43.9		57
Transbaikalia		1.8	76.0	22.2	113
Northern Mongolia		34.7	56.5	8.7	23

Lake Baikal specimens with fused spots become less frequent, and there appear specimens having the typical pattern of *Coccinella divaricata* (Fig. 3 C, seven instead of nine spots). The typical pattern predominates in western Siberia and in Europe (Table 7). The material available from Transcaucasia is very small; it shows, nevertheless, conclusively that in the region just named lives a very light race of *Coccinella divaricata*. Most of the individuals coming from Transcaucasia have only five rather small spots (Fig. 3 D). In southern Turkestan, where one might expect to find the least pigmented race of the species, *Coccinella divaricata* does not occur, or at least has not been found there.

ALLEN'S RULE

There is observed a marked concentration of the scarcely pigmented varieties in Turkestan, Iran, California, Arizona and Mexico. These countries can be described as centers of light forms. The further a region is removed from these centers the darker is the average type of the population found therein. The pigmentation in most species increases roughly proportionally to the distance between a given region and the centers of light

forms. In eastern Siberia, which is very far removed from both the Eurasiatic and the American centers of light forms, a center of darkly pigmented forms is found.

These are the facts. The causation of these facts is, at present, a matter of speculation. Attention is naturally drawn toward the analysis of the geographical, and primarily climatical, conditions encountered in the countries in question. The pigmentation of the Coccinellidae seems to be related to the degree of humidity, and, possibly, to the temperature, prevailing in a given region. The depigmentation takes place in the arid, hot, and desert regions of both Eurasia and America. The humid and cold climate of northeastern Asia produces an increase of the pigmentation. The rôle of humidity seems to be more important than that of temperature since the increase of pigmentation is observed in some species (*Coccinella septempunctata*, see Dobzhansky and Sivertzev-Dobzhansky 1927, also in *Adonia variegata*) in humid but otherwise so different countries as northeastern Siberia, Japan, northern Europe, Abyssinia and Himalaya.

The rule of the depigmentation in arid regions, and of the accumulation of pigment in humid regions, is well known under the name of Allen's rule (or Gloger's rule, see Rensch 1929, or even the erroneous name of Allen's law). This rule holds true in many groups of terrestrial Vertebrata and Invertebrata. The results of Sumner (1920, 1924, 1925) in mice, Görnitz (1923) in birds, Zimmermann (1931) in Vespidae, and Netolitzky (1931) in Carabidae may be quoted here as examples. Together with a series of other rules describing the relations between various characteristics of the organisms and the environmental conditions predominant in their habitat (Rensch 1929, Turesson 1922, 1928, 1930, Alpatov 1925, Netolitzky 1931), Allen's rule represents one of the broad generalizations brought to light by the comparative studies on variation.

It must be, however, emphasized that Allen's rule is nothing more than a statistical rule which holds true only

as far as the majority of the species and the major subdivisions of the continents are concerned, and which can not be stretched to cover all known cases of variation. Thus, the region of the predominance of the light races of Coccinellidae in America includes the entire Pacific Coast from Mexico to British Columbia, although the northern part of this region (from British Columbia to Oregon) can not be classed as arid. The "centers" of light forms in different species include different regions. Thus, the population of *Adonia variegata* inhabiting Chinese Turkestan (the Tarim depression) is nearly as dark as the Siberian population and distinctly darker than the race living in Iran and Russian Turkestan. In *Synharmonia conglobata*, *Coccinella transversoguttata* and many other species the races inhabiting Chinese Turkestan are the lightest known in these species, there being no difference in this respect between the Tarim depression and Russian Turkestan, but a striking difference between the former and Siberia.

ARE THE VARIATIONS IN THE COLOR-PATTERN INHERITED?

The question as to whether the color-patterns are inherited is obviously important for any attempt to evaluate the facts presented above. There exist some experimental data bearing on this question. Johnson (1910) studied the inheritance of the variations in the color-pattern of *Hippodamia glacialis*, *Hippodamia convergens*, *Hippodamia spuria*, *Coccinella novemnotata*, *Adalia frigida* and *Epilachna borealis*. Though his data are unsatisfactory from the standpoint of modern genetics, they leave no doubt that even minor variations in the color-pattern of these species are hereditary. Timberlake (1922) studied the inheritance of three patterns in *Coelophora inaequalis* and of three patterns in *Cheilomenes sexmaculata*. It is clear from the data presented by Timberlake that in the first of these species triple allelomorphs are involved. In the *Cheilomenes* case Mendelian inheritance is also at hand, although the author does not think so.

Lus (1928) made a careful and extensive study on the inheritance of the numerous color-patterns found in the populations of *Adalia bipunctata* and *Adalia decempunctata* inhabiting northern Russia and Turkestan. The existence of at least three loci concerned with the coloration is established in the former of these species. A series of at least eight multiple allelomorphs determines the color-pattern of the elytra and, partly, of the pronotum. Another gene determines the pattern of the pronotum only. A third factor, encountered only in the population living in Turkestan, transforms the black pigment into a brown one. In *Adalia decempunctata* triple allelomorphs for the pattern of the elytra and the pronotum, and an independent gene for the black *versus* the brown pigment were found.

Timoféeff-Ressovsky (Proceed. VI Internat. Congress of Genetics, 2, pp. 230-232, 1932) has published a preliminary account on his work on the geographical races of *Epilachna chrysomelina* F. He concludes that the geographical races in this species differ from each other in complexes of genes; that these genes may be geographically distributed independently from each other; that the races may be characterized by the different frequencies of the different genes in the population; and that not single genes but rather some "harmonic" gene combinations have selective values under a given set of geographical conditions. The results of Timoféeff-Ressovsky are, thus, in a perfect agreement with the views expressed in the present paper.

The hereditary nature of some of the variations of the color-pattern in Coccinellidae may be sometimes proven by indirect evidence. In a multitude of species individuals having very different patterns occur together, in the same locality, and at the same season. There is no indication that the relative frequency of the different patterns in the population undergoes pronounced changes with seasons, though many species have more than one generation per year, and the different generations de-

velop under dissimilar external conditions. As mentioned above, the variability in many species is discontinuous, so that the population is frequently divided into a number of distinct forms, the intergradations being few or absent. These facts taken alone would not afford an entirely conclusive proof of the hereditary nature of variability. Cases are known (in *Forficula*, Diakonov 1926) in which a sharply bimodal variability is due entirely to external influences coupled with a complex norm of reaction. In *Coccinellidae* such phenomena are, however, not known, and, on the contrary, in every case tested experimentally the variations proved to have a genetic basis.

It is, of course, impossible to deny that some of the minor variations in the color pattern may be purely phenotypical. This is especially true for varieties which are never or only seldom found in the same locality. But all the data available argue against the assumption that the phenotypical variation is an important component of either the geographical variability or the variability in populations living in the same region. It is interesting in this connection that the temperature influences applied to pupae and prepupae of various *Coccinellidae* mostly fail to produce appreciable changes in the patterns (Johnson 1910, and unpublished results of the writer).

CONTINUOUS AND DISCONTINUOUS VARIABILITY

As mentioned above, the variability in many species of *Coccinellidae* is nearly or completely discontinuous, that is to say, the intergradations between different color-patterns encountered within a single species are few or absent (see also Dobzhansky 1924). This fact makes it not only convenient but even necessary to consider the geographical variability in terms of the relative frequencies of the various patterns in the different parts of the specific area. Instances of discontinuous variability are met with not uncommonly in different groups of animals and plants (one may mention here only the classical work

of Bateson, 1894). It is, however, known to every one who is sufficiently acquainted with the taxonomy of any group, that cases of continuous geographical variability are also frequent, and in most groups probably even more so than cases of the opposite kind. Races inhabiting remote parts of the specific area may be sharply distinct from each other, and no individuals with the characteristics of the other race may ever occur within the confines of each race. These races may be, however, connected with each other by a series of intermediate races living in geographically intermediate regions, and forming a "sliding passage" ("gleitende Übergänge") between the two extremes (Rensch 1929).

Within the family Coccinellidae both types of geographical races, and also types intermediate between them, are abundantly represented. In *Coccinella sep-*

TABLE 8
SIZE OF THE DISCAL SPOT IN *Coccinella septempunctata*

Region	Mean value	Lim	n
Italy	39.18 \pm 1.23	26 - 50	35
Algeria	38.82 \pm 0.73	25 - 52	50
Archangel	40.62 \pm 0.72	30 - 60	71
Kiev	38.85 \pm 0.54	24 - 50	100
Crimea	37.77 \pm 0.67	25 - 52	100
Transcaucasia	35.55 \pm 0.97	0 - 53	100
North Persia	33.12 \pm 0.95	0 - 45	56
Transcaspia	35.64 \pm 0.74	16 - 50	83
East Bokhara	32.64 \pm 0.70	12 - 51	82
Samarkand	32.43 \pm 0.76	12 - 49	83
East Ferghana	33.06 \pm 0.73	15 - 49	100
Kuldja Province	35.76 \pm 0.64	20 - 50	95
Yenisseisk Province	39.57 \pm 0.76	15 - 58	84
Irkutsk Province	38.97 \pm 0.68	25 - 54	100
Amur Province	45.09 \pm 1.01	34 - 59	34
Maritime Province	43.44 \pm 0.61	30 - 61	100
Korea	47.49 \pm 1.32	34 - 56	29
Ordos	41.31 \pm 1.10	26 - 57	30
Kamchatka	56.13 \pm 0.72	41 - 70	79
Sakhalin	55.92 \pm 1.19	37 - 74	53
Japan	66.22 \pm 1.67	48 - 82	27

tempunctata L. the size of the black spots on the elytra is different in various regions inhabited by the species. Some of the data bearing on the question are presented in Table 8 (according to Dobzhansky and Sivertzev-Dobzhansky 1926). For each region the mean diameter of one of the spots (expressed in units of the eyepiece-micrometer, one unit being equal to 26.3 micra), the amplitude of variation (Lim), and the number of individuals measured (n) are indicated.

The spots are smallest in the populations inhabiting Persia and the southern part of Turkestan. As shown above, this region is one of the centers of light forms. The further a region is removed in any direction from this center, the larger becomes the average size of the spots in the population inhabiting this region. The maximum size of the spots is observed in Japan and the Pacific Coast of Siberia. This, as we know, is the center of the dark forms. It is noteworthy that the discontinuous geographical variation is also observed in *Coccinella septempunctata*. The confluence of the spots is frequently observed in Himalaya and in India, less frequently in Japan, and very seldom in any other region. The disappearance of spots is encountered mainly in Persia and Turkestan.

In *Coccinella novemnotata* (Fig. 3 A, B, E, F) the spots are largest in the population inhabiting the southeastern United States. The size of the spots becomes progressively smaller as one proceeds in the westerly direction; between the Rockies and Sierra Nevada, and also in Oregon, races are found (var. *degener*, Fig. 3 E, and var. *oregona*) having small spots. In middle California (San Joaquin Valley) a race is found in which most individuals have no spots (see the discussion of this point above); a few individuals still possess spots, but the size of these spots is very small.

Some taxonomists are inclined to see a sharp contrast between the continuous and the discontinuous types of the geographical variability, and to consider these two types

as the manifestations of two separate principles, namely the environmental and the germinal variability. A simple analysis may show this contrasting to be entirely fallacious. Provided the racial traits are hereditary (and this is amply proven in a series of cases), the only distinction between the two types under consideration is that between hereditary factors producing minor changes or, else, easily classifiable differences in the external characteristics of an organism. The practice of the geneticists shows that the so-called qualitative differences, especially differences in colors and patterns, are usually inherited on a monofactorial or a simple polyfactorial basis. On the other hand, the so-called quantitative characters, especially those involving the size or the shape of the body or of its parts, are more frequently determined by the interaction of numerous factors. Each of these numerous factors taken alone produces only a small effect, sometimes discoverable only by statistical methods (Sumner, 1930). Here, again, no two distinct types of inheritance are involved. The number of genes concerned in the production of size- and shape-differences may be very small, and color-differences may depend upon the interaction of numerous factors. The distinction between the continuous and the discontinuous types of variation is, then, analogous to that between the simple mendelian inheritance on one hand and the so-called "blending" inheritance on the other. Most geneticists agree nowadays that the latter distinction is entirely spurious.

In cases of discontinuous variability the differences between the geographical races may be expressed in terms of the relative frequencies of genes responsible for the production of various patterns in the different localities. What we are studying is essentially the geography of the genes responsible for the different patterns, and not the geography of the patterns themselves (the "geno-geography," Serebrovsky, 1927). Bernstein (1925a, 1925b, 1930) studied in these terms the geographical dis-

tribution of certain hereditary characteristics in man, mainly that of the blood-groups. It is quite possible to visualize also the continuous geographical variation being represented in exactly the same way (the contrary opinion was expressed among others by Rensch, 1929).

Let us suppose that each of the genes A, B and C increases the size of the spots in *Coccinella septempunctata* by a certain small value. The entire population of this species living in Japan may have the genetic structure AA BB CC; hence, the spots are very large. In Kamchatka only a part of the population may be AA BB CC, and the other part AA BB Cc and AA BB cc; the average size of the spots in the Kamchatkan population must be, then, smaller than in the Japanese population. In Maritime Province individuals of the structure AA Bb cc and AA bb cc may also occur; the average size of the spots must be still smaller. Finally, in Persia and southern Turkestan the majority of the population, or even the entire population, may be aa bb cc, and hence, have very small spots. It is obvious that if a character distinguishing geographical races has a genetic basis similar to that just outlined, the intergradations between these races may be as gradual and "sliding" as the absence of the natural barriers separating these races from each other may allow.

GEOGRAPHICAL AND NON-GEOGRAPHICAL VARIABILITY

Many taxonomists place a considerable emphasis on the distinction between the geographical and the non-geographical variability. The geographical forms are those which predominate in a definite part of the area inhabited by the species, and never or only seldom occur outside of the confines of this part. Such forms are termed subspecies, races, natio, varieties, or "local" forms. The non-geographical forms, termed aberratio, phases, mutants or simply "forms," occur more or less regularly in the entire area of the species, or in a part thereof, but their occurrence does not constitute a clear distinction

between populations inhabiting different regions (Semenov-Tian-Shansky, 1910).

No less importance is ascribed to another distinction between the geographical and the non-geographical forms. The former are characterized by complexes of differences permeating the whole body. The latter differ from each other by often striking but single characters (Rensch, 1929). Jordan (1905) has given an especially concise analysis of the situation as applied to insects. According to him, the geographical forms differ from each other usually, or at least frequently, in the structure of the genitalia as well as in external characteristics, these two kinds of differences being correlated with each other. The non-geographical forms do not possess such correlative alterations in the genitalia.

As far as these ideas represent merely generalized statements of the facts accumulated by the prolonged experience of the taxonomists with their material, they are highly valuable. Indeed, they describe accurately the situation encountered in most groups. The geographical races in Coccinellidae differ from each other as a rule not only in the relative frequencies of the different patterns encountered in the different regions, but also in the size and the shape of the body, the punctuation of the elytra, and other characters the variability of which is continuous. The structure of the genitalia is not, as a rule, a good racial character in Coccinellidae, but some races do differ from each other in the structure of the genitalia. Thus, the shape of the receptaculum seminis is different in the European and the eastern-Siberian races of *Thea vigintiduopunctata*. It is, however, preposterous to consider on these grounds the geographical and the non-geographical variability to be two separate, independent, and perhaps even conflicting processes.

In spite of the scarcity of exact data on the genetics of geographical variability, some of the main features of this phenomenon are clear enough. The geographical races in man and in the domestic animals and plants differ from each other mostly in more than a single gene,

and usually in many genes. The same is true for the geographical races in wild species. Indeed, Sumner (1930) found that the differences between the geographical races of mice are determined by numerous genes. As to the specific differences, which since the time of Darwin have been considered to be merely exaggerated racial ones, the situation is still more clear. Species differ from each other always in many genes.

On the other hand, the differences between the non-geographical forms, however striking they may seem to our eyes, are predominantly monogenic. A change in a single gene may provoke alterations in many characters and in different parts of the body, but one or a few of these alterations are, as a rule, far more conspicuous to our eyes than the rest of them. Hence, the differences between geographical forms involve usually many characteristics, while those between the non-geographical forms are restricted to few characters. This distinction between the geographical and the non-geographical forms seems to follow from the very nature of things. A segregation of a panmictic population into two groups differing from each other in a complex of genes encounters considerable difficulties on its way. As soon as such two groups start to interbreed, the genes concerned will tend to become distributed in the whole population at random, unless some exceptional conditions (found, for instance, in *Oenothera*) prevent it. The formation of such groups becomes more probable if they are at least partly isolated from each other by geographical, ecological or seasonal factors. The geographical isolation seems to be, at least in most animals, the most important kind of isolation contributing toward the splitting of a Linnean species into minor units.

The present analysis of the geographical variation in *Coccinellidae* deals with the behavior of a single character, namely that of the color-pattern. This particular character and this particular group seem to be especially favorable for an exact study because the variability in this case happened to be discontinuous. As far as this

character is concerned, there is no essential difference between the non-geographical and the geographical variation. In fact, they seem to be merely two stages of the same process. The study of the behavior of the characters the variability of which is continuous is for purely technical reasons more difficult. There is, however, no ground to assume that the behavior of the latter kind of characters is in any respect different from that of the discontinuously variable characters.

An analysis of the mechanism of the formation of the geographical races and species ought to begin with a study of the behavior of the single characters distinguishing the different forms from each other. Only subsequently can one study the interaction of the unit-characters in the complex systems representing the types with which taxonomy is primarily concerned.

It would lead us too far to discuss here the possible causes of the differentiation of the species into races distinguished from each other by the relative frequencies of the different biotypes. Three explanations may be mentioned here. First, an originally homogeneous population extending its geographical distribution may become differentiated in accordance with the environmental conditions prevailing in the different parts of the area. Second, various biotypes may arise by mutation; the mutations may be equally frequent in all parts of the specific area, but some of them may become established and others rejected by the natural selection. Different mutations may be favored in different regions. Third, the phenomena observed may be partly accounted for by assuming a hybridization of two or more preexisting races each of which, before the hybridization began, was characterized by a definite color-pattern or patterns. In any case, there is no need to assume that the color-pattern itself is the character with which the natural selection is working. The accumulation or the reduction of the amount of pigment produced in the organism may be responsible for an alteration of its physiological character-

istics, and make it more or less adapted to the environment encountered in a given region.

ACKNOWLEDGMENT

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THE RELATION OF THE INDUCED MUTATION RATE TO DIFFERENT PHYSIOLOGICAL STATES IN DROSOPHILA MELANO- GASTER: I. IRRADIATION AFTER STARVATION¹

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EARLY in the investigation of radiation effects, differences in the susceptibility of tissues and cells were recognized and changes in sensitivity noted. Observations by different investigators are, however, still somewhat contradictory at the present time, and there has been no adequate explanation to account for relative resistance or sensitivity. Such confusion is not surprising, since secondary responses to irradiation greatly complicate the final effect.

In general, rapidly growing tissues have been thought to be particularly susceptible, an observation closely bound up with the fact that during mitosis cells are more easily injured by agents of many different kinds, *i.e.*, temperature, chemicals, etc., as well as by irradiation. As early as 1906 Bergonié and Tribondeau concluded that susceptibility varied directly with reproductive capacity and indirectly with the degree of differentiation of the cells, formulating the first law of radiation therapy. Curtis ('28) observed in work on *Planaria* that undifferentiated formative cells readily succumbed upon exposure, although the animal itself might continue to live for some time.

Cells undergoing division are considered highly susceptible, although there is a wide divergence of opinion

¹ The expenses incurred in connection with the work reported in this paper were defrayed in part by a grant from the Committee on the Effects of Radiation upon Living Organisms of the National Research Council.

as to which is the critical mitotic phase. Vintemberger ('28) concludes that susceptibility gradually increases as mitosis proceeds and then falls sharply at the telephase. Strangeways and Hopwood ('26), studying the effects of x-rays upon mitotic divisions *in vitro*, considered the brief period of organization just before division the high point of sensitivity, while Regaud, in '23, recorded two maxima during the process itself, the prophase and the anaphase.

Cytological studies and genetic experiments both give evidence that some particular part of a cell may be altered while the remainder suffers no perceptible damage. Jannson, in '27, observed profound modifications of the nuclei of cells after exposure—fragmented and misshapen chromosomes, attachment of the division halves by chromatin bridges, etc. Although the continuance of development is impossible where injury is severe, Strangeways and Hopwood ('26) have found that when a cell is irradiated after the mitotic process has been initiated, the division continues in spite of injury. Canti and Donaldson ('26) also studying the effects of radiation on mitosis *in vitro*, found that even a brief exposure to the gamma-rays of radium prevented cells which were about to divide from beginning the process. Unless, however, irradiation is long-continued or very intense, most cells suffer no visible injury but begin to divide again after a short recovery period, giving no evidence of permanent harm. In tissue culture work in which these phenomena have been observed to particular advantage, the temporary check in cell division is followed by a considerable increase in the number of dividing cells. (Regaud, Lacassagne and Jovin, '25.)

This return to activity after relatively slight exposure, and the fact that in tissues or cells under irradiation some part will respond more quickly than another, form the basis of the entire field of those genetic experiments in which lethal and visible mutations, translocations and gene rearrangement have been produced experimentally

in an attempt to solve the problem of the causes of variability among organisms in nature.

That internal conditions do affect the frequency of mutations produced by irradiation is shown also by such observations as the higher rate of induction in mature sperm than in adult females and larval males (Muller, '30). Hanson ('29) and Harris ('29) independently found that the rate of lethals from mature sperm was five or more times higher than from spermatogonia treated in the adult male. Muller ('30) found also a sex difference in the rate of mutation under identical conditions of treatment. It is now generally accepted that this differential behavior of the cells under irradiation is not due to any selective power of the rays, since all parts of the cell and all kinds of cells have about the same power of absorption. Whether the cell is affected or what part of it is altered seems to depend upon the physiological condition at the time of irradiation and on the consequent physical and chemical changes.

With these observations in mind the authors began a study of radiation effects in fruit-flies under such varying physiological conditions as could be produced experimentally: (1) Inanition, (2) complete anesthetization, (3) rapid *versus* slow germ cell formation (comparison of the effects in virgin and in impregnated females), etc.

I. THE EFFECT OF INANITION

(A). Treated males.

Cultures of wild type *Drosophila*, already having minimum food supply, were subjected to overcrowding by increase in the perculture population. In these cultures both the pupae and the flies hatching from them are very small and show obvious signs of starvation. Male flies collected from such cultures were completely starved after hatching for a period of eight hours and were given one hour's exposure to 300 milligrams of radium (gamma radiation only). The alpha and beta rays were excluded by means of a 0.5 mm platinum filter which at the same

time allows the passage of 78 per cent. of the gamma radiation. Secondary radiation was reduced to a minimum by filtration through a layer of Columbia paste 1 cm in thickness. The flies were enclosed for treatment in thin gelatin capsules. Following treatment these starved males were mated to well-fed C1B females of the stock.

The results obtained differ significantly in two respects from those of unstarved treated controls: sterility of the

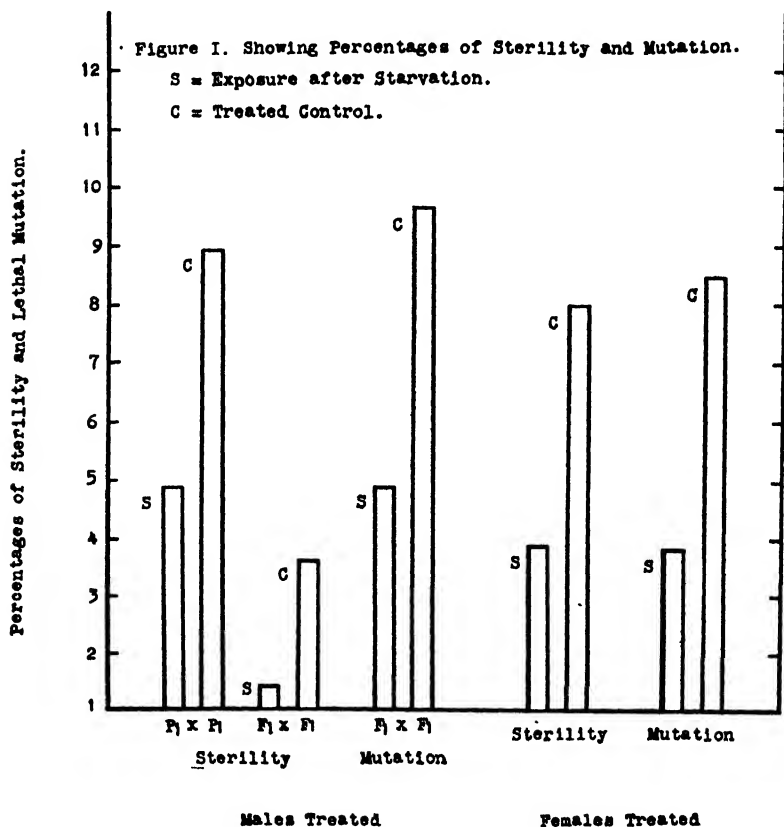
TABLE I
SHOWING THE PERCENTAGES OF STERILITY AND LETHAL MUTATION FOLLOWING IRRADIATION OF *DROSOPHILA* AFTER STARVATION AS COMPARED WITH UNSTARVED CONTROLS

Generation	Exposure after Starvation	Total No. Cultures	Treated Control	Total No. Cultures	Difference	Significance
Treated Males						
<i>Per cent. of Sterility</i>						
$P_1 \times P_1$						
	4.8036 ± 0.2845	500	8.9887 ± 1.4451	178	-4.1851 ± 1.3862	$3.01 \times p.e.$
$F_1 \times F_1$						
	1.3333 ± 0.1997	1500	3.6923 ± 0.4988	650	-2.3590 ± 0.5373	$4.31 \times p.e.$
<i>Per cent. of Lethal Mutation</i>						
$F_1 \times F_1$						
	4.8814 ± 0.3716	1475	9.7444 ± 0.7980	626	-4.8630 ± 0.8977	$5.42 \times p.e.$
Treated Females						
<i>Per cent. of Sterility</i>						
	3.8000 ± 0.3262	1370	8.000 ± 0.4724	1500	-4.2000 ± 0.5740	$7.32 \times p.e.$
<i>Per cent. of Lethal Mutation</i>						
	3.8113 ± 0.3399	1318	8.4782 ± 0.5085	1380	-4.6669 ± 0.6016	$7.75 \times p.e.$

Total number of initial single-pair matings on which these figures are based: Series of Treated Males, 3,143; Series of Treated Females, 3,525. Sterility tests are additional to these figures.

parent and F_1 generations and lethal mutation rate. Mortality in the parent generation, based on the number dead by the second day after exposure was high: 11.40 per cent. ± 0.958 in a total of 500 P_1 cultures, as compared with no mortality in the controls. This high rate of post-irradiative mortality is indicative of the severity

of the starvation, 11.40 per cent. being unable to survive the combined effects of inanition and irradiation. The percentages of sterility in both generations and the rate of lethal mutation were significantly lower. As in the experiments dealing with equivalent dosages (paper in press at the time of this writing), these sterility values are absolute, representing complete sterility. Sterility tests were carried out in the same manner as before. It is interesting to note that here also, as in the proportionality experiments, the amount of sterility occurring in the second generation is approximately one half that in the treated generation. In Table I these percentages are compared and their differences are given. Figure 1 shows the results graphically.



(B). Treated Females.

First generation bar-eyed females of the cross C1B by wild were selected for treatment. Such females, having one x-chromosome carrying the C1B factors and one x-chromosome the wild-type are heterozygous for the lethal character and when mated to the wild type give one half the usual number of males in the F_2 generation. If a new lethal mutation were induced in the chromosome bearing the wild-type characteristics, the other half of the sons would inherit it and also fail to appear. Preliminary experiments demonstrated the occurrence of an induced lethal in this x-chromosome.

These F_1 females were subjected to starvation in exactly the same way as the males previously described and exposed to the same dosage, 300 milligrams for 1 hour. The results confirm those obtained earlier with wild-type males where the lethal is carried by the F_1 female and detected in the second generation. Here again, both the sterility and the mutation percentages are significantly lower than those in unstarved treated controls.

Concerning the comparative effects on the two sexes, Table II reveals that the mortality, sterility and muta-

TABLE II
SHOWING SLIGHT DIFFERENCES IN THE RESULTS OBTAINED WHEN MALES AND
FEMALES ARE IRRADIATED UNDER IDENTICAL CONDITIONS
OF TREATMENT

Percentage of	Males Treated	Females Treated	Difference
<i>Treated Control</i>			
Sterility	9.9887 ± 1.4451	8.0000 ± 0.4724	0.9887 ± 1.5133
Mutation	9.7444 ± 0.7980	8.4782 ± 0.5085	1.2662 ± 0.9462
<i>Exposure After Starvation</i>			
Mortality	11.4000 ± 0.9586	8.6666 ± 0.4900	2.7334 ± 1.0724
Sterility	4.8036 ± 0.2845	3.8000 ± 0.3262	1.0036 ± 0.9570
Mutation	4.8814 ± 0.3716	3.8113 ± 0.3399	1.0701 ± 0.5036

tion values are consistently less when females are treated, although not significantly so. The differences

with their probable errors are given. Muller ('30) has found some indication of sex differences, the "rate of induction in mature sperm" being "higher than that in adult females. . . ."

Evidence from a number of sources shows that the cells of starved tissues undergo division less and less frequently as starvation continues and then remain in a resting condition until degenerative changes set in. This mitotic inactivity accompanying inanition has been observed particularly in work on transplantation and grafts, and also in tissue culture work, where normal tissue culture cells are deprived of nutritive fluids. Since cells initiating the division cycle very infrequently and those in the resting phase are less susceptible to the effects of radiation, it seems logical to suppose that the marked decrease in radiation effectiveness observed in the starved tissues in these experiments might be attributed to a decrease in mitotic activity.

SUMMARY

In two series of experiments, one in which male *Drosophila* were treated and one in which females were treated, flies hatched from cultures having minimum food supply and completely starved for a period of 8 hours preceding treatment were given an exposure of 300 milligram hours, gamma radiation only. The results obtained differ significantly in two respects from those in unstarved treated controls: sterility of the parent and F_1 generations and lethal mutation rate. Mortality in the starved parent generation, based on the number dead by the second day after exposure, was high as compared with no mortality in the treated controls. The percentages of sterility in both generations and the rate of lethal mutation were significantly lower. Possibly these results may be explained by the fact that the cells of starved tissues are less actively dividing and perhaps, therefore, less susceptible to the effects of radiation.

The authors take this opportunity to acknowledge, with

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² In other papers of this series items of the literature list will be referred to according to the above numeration.

AN EMBRYOLOGIST'S CONCEPTION OF VERTEBRATE PHYLOGENY

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THE EMBRYOLOGICAL DATA

For a number of years, the writer has been interested in a phylogenetic study of vertebrate embryology. That embryology throws considerable light on various problems concerning the interrelations of the vertebrate classes has, of course, been recognized for years, but I can not help feeling that some of the strongest lines of evidence to be drawn from this field have been consistently ignored or overlooked. In the following pages, I propose to offer a revised phylogeny which better harmonizes the known embryological facts, and which is at the same time consistent with comparative anatomy and paleontology. I have no doubt that I will be criticized for the radicalness of my departure from assumptions which have passed unquestioned for so long that they have acquired a considerable odor of sanctity, but I can not allow reverence for the ancient to prevent my calling attention to serious inconsistencies in the accepted scheme.

Of the text-books on general zoology, vertebrate zoology or evolution, the great majority of those which discuss phylogeny describe or diagram the vertebrate family tree as being like the scheme drawn in Fig. 1. That is, the elasmobranchs are considered as the most primitive group (omitting the cyclostomes from the list of true vertebrates); these give rise to an ascending series of ganoids, amphibia and reptiles in the order named, with birds and mammals branching independently from the reptilian stock. The teleosts are regarded as a side branch from the ganoids, lung fishes either as a side branch or as a precursor of the amphibia.

¹ Contribution No. 230, from the Zoological Laboratory.

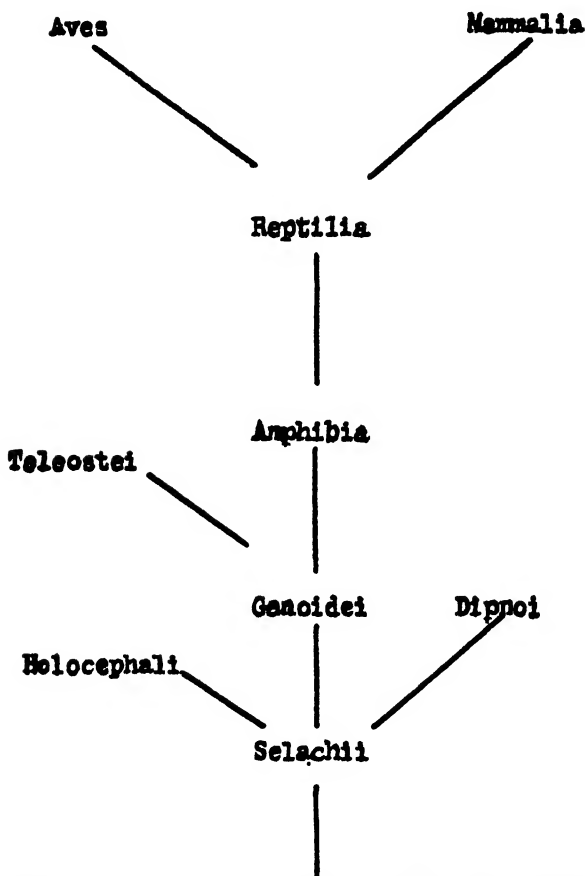


FIG. 1. Vertebrate phylogeny according to the generally accepted scheme found in most zoological texts.

There seems to be more diversity of opinion among paleontologists as to the relations between the various groups of fishes, although the greater part of the texts I have examined in this field follow in general the above scheme. Most of them look upon the lung fishes as being coordinate with the ganoids; but practically all of them agree with the zoological books in placing the elasmobranchs as the ancestors of the fishes and in deriving amphibia from a ganoid or dipnoid stock.

Now let us examine the eggs and ways of development of these groups, and see if they will fit into the above

scheme of evolution. The most primitive ova, as regards yolk content and cleavage, are found in three distinct groups: ganoids, lung fishes and amphibia (omitting, of course, the specialized caecilians). There is not much to choose from among these three groups; each group shows as much variation among its members as there is among the groups as units (see Fig. 2). All three have

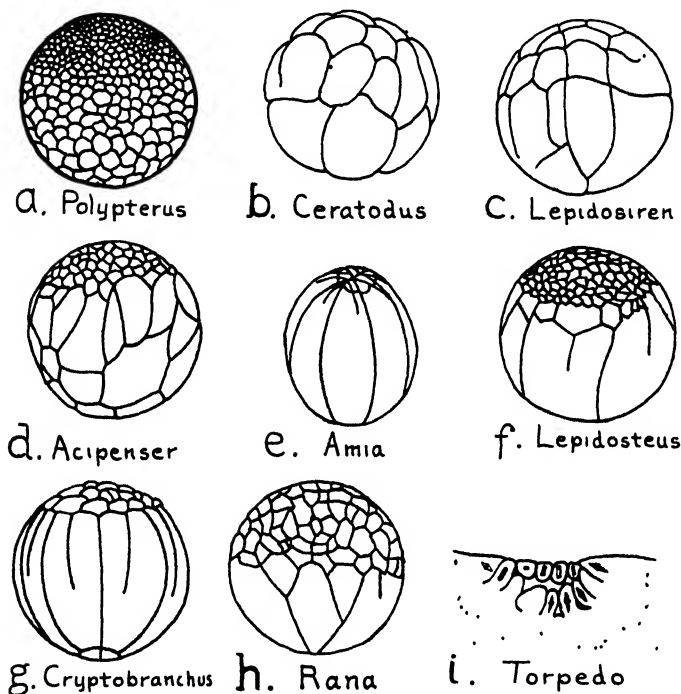


FIG. 2. Cleavage stages of various fishes and amphibia, to illustrate points discussed in the text. All surface views except i, which is a cross-section. Redrawn after: a—Budgett, b—Semon, c—Kerr, d—Dean, e—Whitman and Eycleshymer, f—Balfour, g—Smith, h—Morgan, i—Ruckert.

ova which are telolecithal, but in which the amount of yolk is not excessive, not enough at least to prevent total cleavage. The remarkably similar cleavages of the three types are followed by gastrulation processes which again are but minor variants of a single type (Fig. 3). Following this, in each case, a larva is produced which we may speak of as the tadpole larva. These tadpole larvae

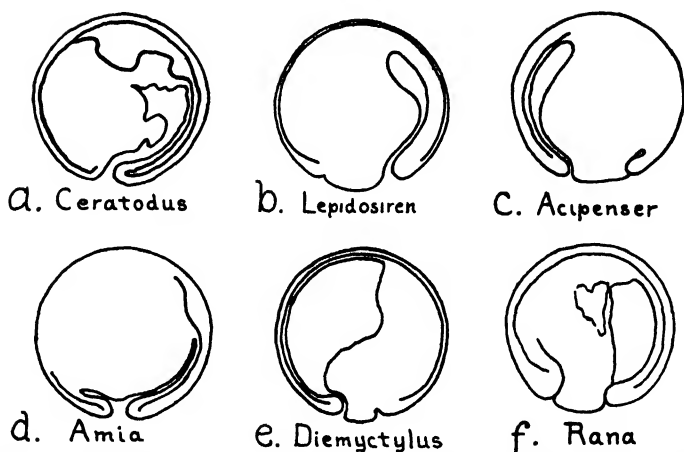


FIG. 3. Sections through gastrulae of dipnoids, ganoids and amphibians, to show their essential similarities as pointed out in the text. Redrawn after: a—Semon, b—Kerr, c—Salensky, d—Dean, e—Jordan, f—Brachet.

vary somewhat from group to group, with, however, only one fundamental difference separating any of them. The general body form is similar in all of them; they all have external gills and diphyccercal caudal fins; and they all have paired appendages which are early used in salamander and Polypterus to balance the body on the bottom when the larva is not swimming by means of the caudal fin, but the appendages of the amphibian larva show toes, while those of the other groups remain simple lobes with fin-fringes around their edges (Fig. 4). Thus early in development does the differentiation into finned and footed lines make its appearance.

Now let us consider the embryological fitness of the sharks to be ancestors to the amphibian-ganoid-dipnoid fraternity. The ganoid egg is representative of all three groups; consequently we shall refer to it alone. Similarly, whatever is said about the embryology of the shark applies equally well to all selachians and to the Holocephali. The ganoid egg is mesolecithal; the shark egg is excessively yolk-laden—so much so that the shark egg rivals that of the bird in its enormous size and exaggerated telolecithality. The ganoid egg cleaves completely,

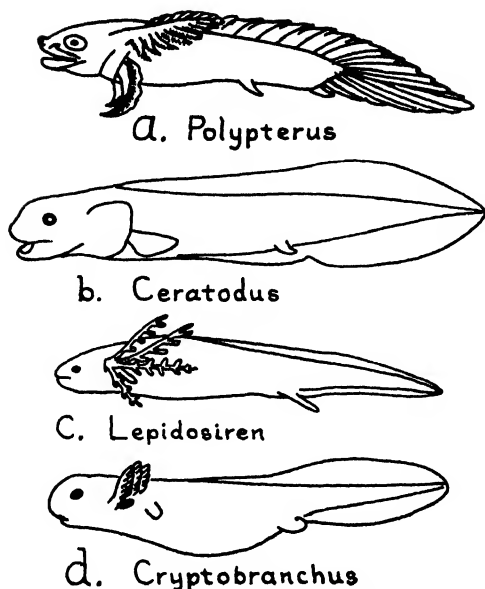


FIG. 4. Tadpole larvae of crossopterygian, dipnoids, and amphibian. Redrawn after: a—Budgett, b—Semon, c—Kerr, d—Smith.

even though more or less unequally; the shark egg has a specialized type of meroblastic cleavage. The ganoid egg gastrulates largely by the epibolic growth of a dorsal blastoporal lip which eventually encloses the more sluggish vegetal pole; the shark blastula gastrulates by involution of the margins of a germinal disc. Concrescence in the ganoid plays but an insignificant rôle, forming only the caudal portion of the embryo; in the shark the greater part of the embryo is laid down by the concrescence of the germ ring. If there is any rhyme or reason to embryological processes, then the ganoid has the simpler type of development, and it is impossible to look upon them as having been derived from selachian ancestors.

It may be urged that the ganoids have lost the yolk possessed by their ancestors and have simplified their development accordingly. The answer to that objection is to be found in the placental mammals. Here is a group descended from ancestors which possessed heavily yolk-

laden eggs, but which at the present time has returned to an almost yolk-free condition. Now in the cleavage, gastrulation and notogenesis of the placentates there is an endless succession of peculiarities which show their simplicity to be not primitiveness but a secondary simplification of an original specialized condition. Even if we lacked the connecting links afforded us by the Prototheria and marsupials, there would still be no possible question as to the derivation of the placentates from a telolecithal, meroblastic-egged ancestor. The ganoids show no evidences of a comparable simplification of development. Their conditions are truly primitive, not derived.

Another argument against the significance of the more primitive ganoid embryology will be the suggestion that our modern sharks have become specialized in their embryology, while the Paleozoic sharks may have had a simple type of development that might well be ancestral to that of the ganoids. This objection is of course more difficult to refute than the first. Paleoembryological research, at a distance from the Devonian to the present, can claim scarcely more substantiality than a spiritualistic seance. For all that we can prove, Devonian sharks *may* have laid small, holoblastic ova, and all their surviving descendants *may* have developed a single type of specialized development, but there are one or two other lines of evidence which cast doubt on this hypothesis and tend to strengthen our original assumption. One of these is the fact that holocephalan embryology is essentially the same as selachian embryology, although the two lines diverged during the Devonian, thus making it probable that the present type of development must have already been established at that time. We will consider some other evidences in succeeding paragraphs.

Having proceeded thus far, let us see how we can reconstruct our vertebrate tree to harmonize the facts brought out in the preceding pages. The result is shown in Fig. 5. As our starting point we will fall back upon

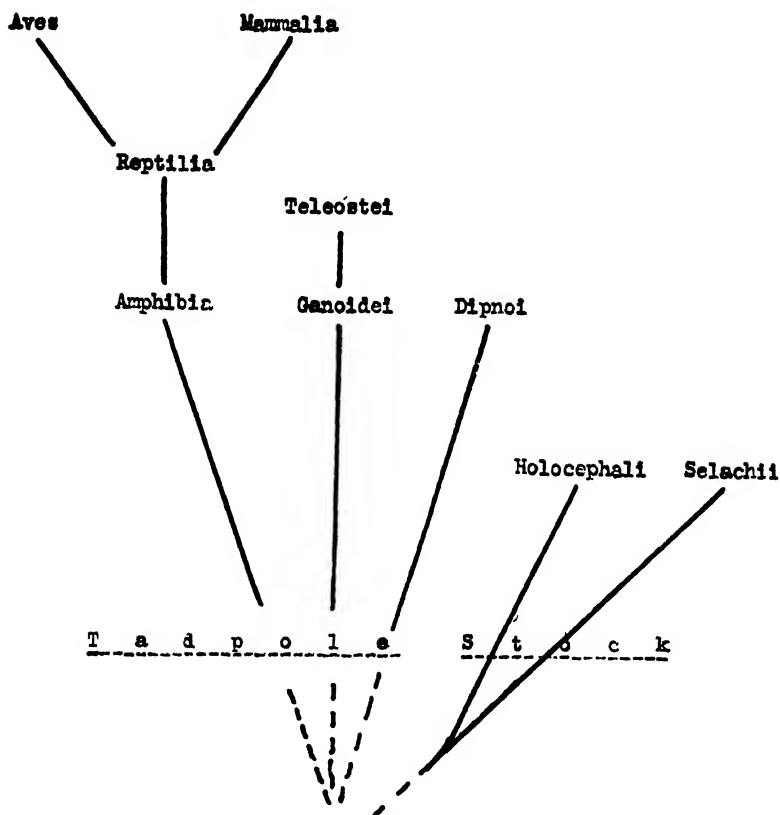


FIG. 5. The phylogeny of the vertebrates according to the outline suggested in this paper.

the Biogenetic Law and postulate an ancestor (or rather an ancestral class) which developed from a mesolecithal, holoblastic egg into a tadpole-like adult with swimming caudal fin, paired appendages used for balancing on the bottom and external gills. This form still appears in the ontogeny of amphibian, ganoid, and lung-fish, and these groups are to be looked upon as descendants of this "tadpole" ancestor. Since, however, there is no stage comparable to the adult ganoid or lung fish in the development of amphibians, the larval stage of one being homologous to the larval stages of the others, neither ganoid nor lung fish can be looked upon as ancestral to the Amphibia. These three groups are of coordinate

rank, evolved to somewhat similar degrees of specialization but along different and distinct lines of evolution.² Paleozoic ganoid and stegocephalian were cousins, not grandparent and grandchild.

Even back in the distant days when ganoid and stegocephalian had not yet made their appearance, their future début was forecast by the splitting of the "tadpole" ancestors into two factions, one of which "clove the hoof," while the other was content to fringe it with fin rays and membrane. The two types of appendage are so distinct in their earliest embryological stages to-day that this schism must have taken place very early in the evolution of the stock. The pentadactyl line formed apparently a homogeneous group which gave rise to but a single line of evolution. The opposition was less unified and their descendants have branched out in several directions. We have already mentioned ganoids and lung fishes which evolved along somewhat similar lines, while some of the ganoids almost paralleled early amphibian evolution, with the exception of the form of the appendages. Sometime in the early history of the "tadpole" group, a stock split off and, modifying profoundly its embryology by the accumulation of yolk and the entailed changes in cleavage and gastrulation, became the sharks, while an offshoot from it, following a somewhat similar line of evolution, formed the chimeras. The embryology of the shark has of course been profoundly modified by the suppression of larval development. It is interesting to note, however, that it still shows, while in the egg or the uterus, external gills and a diphyccercal tail. I interpret this to indicate a descent from some level of the "tadpole" stock.

² It should be recognized that the change from larval *Polypterus* to adult is fully as much of a change as that involved in the growing up of, let us say, a larval *Necturus*. We are too used to thinking of the striking metamorphosis that takes place in the anura and the terrestrial salamanders as being typical of the whole class. A great part of the phenomena of metamorphosis has been acquired since the amphibia became such definitely. The perennibranch salamanders alone show the primitive condition.

Above the level of the amphibian-ganoid-lung fish trimvirate, we are once more on solid ground where there is but slight difference of opinion as to the relationships of the groups. The teleosts are almost universally admitted to be an offshoot from the ganoids. They are a side branch which has become highly specialized in egg and embryology as well as in adult structure, although we can see in *Amia* and *Lepidosteus* a foreshadowing of the teleostean type of development. From amphibia we have had the reptiles arising, and their embryology can likewise be easily derived from that of the Amphibia. The bird represents merely an extreme specialization and exaggeration of the reptile type of development; the mammals present the reverse process, a simplification of the reptilian type, which has, however, superimposed specialization on top of specialization rather than bringing about a return to primitive conditions.

EVIDENCES FROM THE FOSSIL RECORDS

We come now to the paleontological evidence. Apparently the first fossils of true fishes (that is to say, omitting the aberrant Ostracoderms) are those of primitive acanthodian sharks from the Upper Silurian, although it is sometimes stated that ganoids also made their appearance in this period (Zittel, "Text-book of Paleontology," Vol. 2, p. 114). In the Devonian, we find two primitive groups of selachians, Holocephali, crossopterygian and paleoniscid ganoids, lung fishes and arthrodires. Also in the Devonian we have the footprint of an amphibian, and that footprint implies a form which had not only evolved far enough to have a true foot but which walked on it. There seems to be good fossil evidence to show that the more primitive of the Paleozoic amphibians were not land forms but were aquatic, with permanent gills and weak appendages. The beast which walked across the Devonian mudflat must then not have been a primitive form, but must have been already fairly well evolved toward the stegocephalian type.

We are accordingly forced to conclude that ganoids, lung fishes and amphibians came into existence at about the same time. We are dealing here with the splitting of an ancestral stock into three coordinate and contemporary lines, rather than with a series of ascending and related forms which follow each other in time. This, of course, is what we had already concluded from the embryological evidence, and we are strengthened in our belief that the phylogeny expressed in Fig. 5 is essentially correct.

The sharks offer more of a problem. The first to appear are the extinct acanthodian (spiny-finned) sharks which are suspiciously ganoidean in many ways. They were in fact classified as ganoids for many years. Woodward, in the revision of Zittel's "Paleontology" places them in the Selachii, but as a separate order equivalent to all living sharks and rays. In the Devonian we have the appearance of a form belonging to another extinct selachian order, Cladoselache, besides representatives of the Holocephali. True sharks do not appear until the Mississippian; they are thus preceded in time of appearance by crossopterygians, lung fishes and stegocephalian Amphibia.

In view of the above facts we can see what should have been evident from theoretical considerations, that the group of modern elasmobranchs can not possibly be ancestral to ganoid, dipnoid or amphibian. Nor is it possible to think of the most primitive selachian as that ancestor. Cladoselache is recognized by most specialists as the most primitive type, but so far as the record goes it was not the earliest. The only group which appeared early enough to be ancestral to the ganoids is the Acanthodii, and that only if the ganoids did not appear in the Silurian, as they are said to have done. Even granting the priority of the acanthodians, it is to be remembered that they are almost as much ganoid as selachian. Once again we would seem to have a divergence into two types instead of a sequence of one from the other.

It is perhaps significant in this connection to point out that the dominant and most highly diversified groups in the Devonian were the ganoids, lung fishes and arthrodires. The sharks were insignificant in numbers and in versatility, not attaining a dominant position until the Mississippian. While this is not proof, still it does offer presumptive evidence that the higher groups could not have been mere newcomers, recently descended from the selachians, but must have been in existence for a long time, probably as long as had the elasmobranchs themselves.

COMPARATIVE ANATOMY AND THE PAIRED APPENDAGES

The relations between comparative anatomy and the suggested phylogeny can be considered from two angles. In the first place, we should of course consider whether the facts of comparative anatomy are such as to make the proposed relationships impossible. Secondly, the theory should be examined to see if its acceptance would enable us to explain any points which, under our present scheme, are obscure or unsatisfactory. It is impracticable to discuss the first point in any detail in this paper; I shall merely make a general remark or two about our interpretation of so-called "primitive" characters. Under the second heading, I shall point out the implications of the theory with regard to the evolutionary relationship between the two kinds of paired appendages, a question which has been a stumbling block for many years.

With regard to the first point, it is usually assumed that the sharks must be ancestral types because of their possession of a large number of characters believed to be primitive, such for example as the cartilaginous skeleton, exposed gill clefts and lack of air bladder. That these are primitive characteristics is, I think, beyond question, but I think that it is questionable as to their persistence in the shark implying that the selachians must therefore be ancestral. It may with equal plausibility be interpreted as meaning that the sharks are an

unprogressive side branch which has retained many primitive characters, while more plastic and progressive stocks have discarded these and so evolved into the higher groups. We see instances where groups which are quite evidently not ancestral have retained primitive or embryonic characters, for example, the cartilaginous skeleton and persistent notochord of the sturgeons. The cartilaginous skeleton and exposed gill slits of the shark are embryonic conditions which have to be passed through before reaching the higher conditions of adult teleostomes; their presence is not an evidence of the ancestral position of the shark, but merely shows that in those respects the shark has not evolved far. Undoubtedly, the forerunners of shark and of ganoid were both cartilaginous, but that is far from saying that these forerunners were identical. They may have been as unlike as are present-day shark and sturgeon. The fact that the shark has not improved its skeleton while the ganoid was developing bone may leave it more primitive, but it does not thereby turn it into a forefather.

As regards the other side of the picture, it would seem that an acceptance of the idea that the stocks we have been discussing have arisen as independent lines will remove one historic stumbling block from the path of the comparative anatomist. No longer will he have to attempt the thankless task of attempting to derive the cheiropterygium from the ichthyopterygium, in the face of both embryology and paleontology insisting that a foot is a foot and a fin a fin from the beginning on. Obviously, of course, there must have been some common starting point for the two kinds of paired appendages, but this ancestral structure must antedate the development of a definite skeleton. On no other ground can I conceive of at least three types of skeleton being evolved with apparently no transitional conditions between one type and the others.

These types are as follows: First, we will name the pentadactyl cheiropterygium, developed by the pre-

amphibian line and passed on by them to all land vertebrates; second, comes the shark-crossopterygian type of fin which has three basals and many radials. From this, by reduction of parts, has been derived the types found in other ganoids and in teleosts. Last of the three is the biserial fin of the lung fishes with its central axis and paired rows of radials. It is interesting to note that one group of Carboniferous elasmobranchs (*Pleuracanthus*) has this same type of fin. One of these fin types can be derived from the other in theory; actual transitional fossils are lacking. Neither embryology nor paleontology shows any intermediate condition between either fin type and the pentadactyl limb, and the multiplicity of theories as to the mode of evolution from one to the other is ample evidence of the lack of any satisfactory hypothesis as to how the change might have occurred.

If instead of holding that the Amphibia have evolved from fishes with a fin skeleton already developed, we conceive of both amphibia and crossopterygian as having diverged as independent lines at a time when the skeleton of the paired appendages had not been definitely established and specialized, then the necessity of trying to account for one in terms of the other vanishes. Dean considers that the parallel fin rays of *Cladoselache* represent the primitive type of skeleton, although this group of sharks is not known until late in the Devonian. It would doubtless be possible to derive both cheiropterygium and ichthopterygium from that type of appendage, and although I do not believe that *Cladoselache* can be looked upon as being primitive enough to serve as an ancestral type to the various lines of vertebrates, it may yet serve to indicate the sort of unspecialized appendicular skeleton which gave the starting point for both foot and fin. At any rate, if our hypothesis is true we can look upon the foot of the tetrapods as having been derived, not from any type of fin existing to-day or (with the possible exception of *Cladoselache*) even from any known fossilized type, but from some primitive precur-

sor of both foot and fin that was so undifferentiated at the far distant date when the two lines diverged that no homologies can be traced to-day between the two kinds of appendages into which that primeval lateral flap of skin has evolved.

THE HABITAT AND EVOLUTION OF THE "TADPOLE" STOCK

It has been suggested for some time (*e.g.*, Chamberlain, 1900) that the commonly accepted idea of the marine origin of the fishes might be wrong. Chamberlain pointed out that the strata which yielded the earliest vertebrate remains were not typical marine deposits but were shore-line deposits, or, in many cases, of river or swamp origin. The inclusion of some fossils in marine strata, particularly in littoral deposits, could be explained on the basis of their being washed out to sea, and the richest beds, such as the Old Red Sandstone, were almost surely of fresh-water deposition. At the present time, it is pretty generally agreed that ganoids, lung fishes, arthrodiros and stegocephalians are usually found fossilized in circumstances which argue a fresh-water habitat for them. The same seems to be true of acanthodian and pleuracanth sharks, which, as we pointed out elsewhere, bear striking points of similarity to ganoids and lung fishes, respectively. The other sharks (possibly excepting *Cladoselache*) and the chimaeras seem to be marine. It is a highly significant fact that among living species all aquatic amphibia, all lung fishes, *Polyp-terus* and most ganoids are fresh-water inhabitants. Some sturgeons and one of the gars have become partially adapted to salt water, but they still return to fresh water to spawn.

On the basis of these facts, I shall speculate a little on the possible early differentiation of the vertebrate stock. At a very early date, prior to the evolution of the "tadpole ancestor" discussed previously, the vertebrate progenitor must have been a form which looked something

like a young shark embryo. It would have had an elongated form, with a well-developed diphyccercal caudal and with lateral folds which had already begun to enlarge locally to form rudimentary paired appendages. The gill clefts were exposed, and the gill filaments were external. In its early development this pre-vertebrate doubtless was somewhat more primitive than our present-day salamanders. I am inclined to believe that this creature must have lived in fresh water; if not, then some of its members must have very early migrated into fresh water, for in sluggish streams, in swamps or in lakes it evolved somewhere near the opening of the Paleozoic into the "tadpole" stage.

In this evolution we can be sure of certain changes. For one thing, the lappets of the lateral folds developed into distinct paired appendages and these developed supporting tissues which in different groups became fin-like or hand-like. The external gills changed in form; from being separate filaments they took on a branching, tree-like arrangement such as we see to-day in young salamanders. A little later the stock began to protect the gills by skin folds, and so initiated the operculum. The most important change was one which made possible the evolution of terrestrial life. The lagoons and swamps in which these fresh-water forms developed must have been subject to fouling or partial drying up which threw a severe strain upon the gill-breathing mechanism of the tadpole stock. To supplement the gills, and to tide them over critical periods when the lack of oxygen became acute, the animals early developed diverticula from the fore gut into which air could be gulped and from which the oxygen could be picked up by the vascular walls. We are justified in this assumption by the universal occurrence of an air bladder connected with the esophagus in all primitive living fishes above selachians. When we remember that there exist in living ganoids and lung fishes all combinations of dorsal or ventral ducts, single or bifurcated diverticula, and smooth or sacculated linings

of the diverticulum it is easy to see that there is no morphological basis for differentiating between lung and air bladder. As for the physiological likenesses, it is known that lung fishes, *Polypterus* and *Lepidosteus*, are able to live out of water for hours, the air bladder functioning as an efficient lung so long as the air breathed is moist. Probably any of our hypothetical "tadpoles" could have evolved into air-breathing forms, so far as the respiratory apparatus was concerned. The determining factor must have been the possession of pentadactyl limbs which made the emergence onto land possible.

At some time in the early evolution of the "tadpole" group a stock split off which was destined to give rise to the elasmobranchs. This divergence must have occurred at a time when the skeleton of the paired fins was already being established, for the skeletal homologies between crossopterygian and selachian fins are too exact to be accidental, but before the evolution of the swim bladder had made any headway. Since the most primitive sharks seem to have been of fresh-water habitat, it would seem that the early evolution of the stock probably took place in fresh water. The group must have penetrated very rapidly into salt water, however, and with the appearance of the true sharks in the Mississippian it became definitely marine.

The successful assumption of marine life by the shark tribe was contingent upon, and probably forced by, a modification of their embryology. The jelly-like egg masses of the amphibia or the small and separate ova of the ganoids will develop successfully in sluggish streams or in quiet and shallow waters; they would have no chance of living in the rough surf of a seacoast. Before they could migrate permanently into the ocean the sharks must have developed, at least to some extent, the protective membranes and egg-laying habits that are successful to-day, and along with this they likely showed the beginning of the yolk accumulation that is so characteristic of the modern species. Judging by present-day conditions,

however, this specialization of the egg with its accompanying lengthening of development spelled the doom of the group as fresh-water forms. Our frogs and salamanders rush through their embryology with frantic speed to complete their development before the pools in which they spawn dry up or become too foul in the shallows for them to survive. A group which was spinning its embryological development out to 150 or 200 days would be forced to find a new environment in which conditions suitable for development prevailed practically the year round. Thus the building up of the yolk content, the lengthening of development and the evolution of the egg capsule not only made it possible for the sharks to become marine but practically forced them to do so.

As a result of the migration into oceanic waters, the selachians have never evolved an air bladder. We have already indicated above that this structure was developed to supplement gill breathing in the stagnant swamps and poorly aerated pools in which the "tadpole" fraternity were evolving. It is thus primarily an organ of respiration, as it is exclusively in the tetrapoda; its hydrostatic function has been secondarily assumed. With the sharks' migration into the well-oxygenated ocean waters the reason for the acquisition of such a structure vanished, and it has never evolved in this group. Or, it may be that the failure of the sharks to develop this accessory organ of respiration was a potent cause of their expulsion from their ancestral fresh-water home.

CONCLUSIONS

(1) The eggs, cleavage and larvae of the ganoids, dipnoids and non-caecilian amphibians are strikingly alike. These three groups are to be looked upon as diverging branches from a common stock. Ganoids are coordinate with amphibia, not ancestral to them.

(2) The occurrence in ganoids, dipnoids and amphibians of a common type of tadpole larva indicates the

former existence of a class of animals resembling these living larvae. This group was ancestral to the three modern groups mentioned.

(3) The highly specialized egg and development of the selachians make it impossible to look upon this group as being ancestral to the simpler type represented by the ganoids, etc. It seems more likely that the sharks arose as a diverging branch from an early level of evolution of the "tadpole" ancestor.

(4) Paleontology strongly supports the conclusions based upon embryology. Ganoids, lung fishes and amphibians of far from primitive types appeared simultaneously in the Devonian. The first selachian fossils are found in the Upper Silurian; these, however, are not typical elasmobranchs but are a type intermediate between shark and ganoid and referable to either group, or neither, with about equal probability. A reasonable interpretation of the fossil record would seem to be that an early fish-like form, not referable to any living group, split into two lines, of which one became the sharks, the other again branching three ways to give rise to ganoids, lung fishes and amphibia.

(5) The foot of the tetrapoda has not been derived from a ganoid-like fin, since we conclude that ganoids are not ancestral to amphibians, but both kinds have been derived from a much simpler primitive appendage whose skeletal elements had not taken on any specialized arrangements. The skeletons of foot and fin can not therefore be homologized.

(6) The early evolution of the vertebrates took place in fresh water, the selachian stock migrating into the oceans. The forms which remained in fresh water evolved an air bladder as an accessory organ of respiration, and the stock which developed walking legs and was able to leave the water evolved the air bladder into true lungs. The sharks may have been forced to a marine environment partly because they failed to provide this means of supplementing the gills, or they may have

failed to develop the air bladder because of their migration into the well-aerated seas. The specialization of the selachians' embryology likewise played an important rôle, making it possible for them to become marine and making it impossible for them to continue breeding in fresh water.

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THE FISHES OF LAKE LANAO: A PROBLEM IN EVOLUTION¹

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ORGANIC evolution, the development of new forms of life from previously existing stocks, is a fact taken for granted by all scientific men. Yet many fail to recognize that the evolution of new species of animals and plants is a continuous operation not confined to past geological ages, but actually going on during the present era.

Students of lichens have long recognized that the evolutionary development of those strange organisms is now actively in progress in numerous cases. The most marked illustration is the strongly differentiated lichen genus *Cladonia*. This group contains many polymorphic and intergrading species, each with a host of varieties, which only a little study shows are now in process of evolution. It is therefore no wonder that *Cladonia* comprises some of the most puzzling forms known to the student of plant life and is perhaps the best example extant of evolution under natural outdoor conditions here and now.

For more than a dozen years it has been my lot to devote a good deal of time to exploring and studying the plateau of Lanao, in the great island of Mindanao. While engaged in a preliminary reconnaissance of the aquatic life of Lake Lanao, for the purpose of helping improve the food supply of the more than 90,000 Marinao living around the lake, I discovered a unique endemic fish fauna.

Lake Lanao is a sheet of open water covering 145 square miles, lying at an elevation of 2,100 feet. For much of its extent the lake is comparatively shallow;

¹ The Cyprinidae of Lake Lanao are described by Herre in the *Philippine Journal of Science*, Vol. 24, 1924, pp. 249-306 and Vol. 29, 1926, pp. 499-502, and in *Copeia* for October, 1932, pp. 140 and 141.

along its northern, eastern and southeastern shores are great areas of shoal water with an average depth of four to ten meters. Along the western shore it is relatively deep and there is deep water from the outlet to the southern shore directly opposite. A half mile from the outlet the lake is 150 feet deep, and this depth increases as one goes toward Bayong, 18 miles away on the southern shore. Near Bayong the lake has a depth of 900 feet, according to the military cable survey.

At the northwest corner of the lake is its outlet, the torrential Agus, which rushes headlong to Iligan Bay 14 miles away. A few kilometers from the sea the Agus leaps over the Maria Cristina Falls, a jump of 65 meters.

Mindanao was originally not less than five rather large islands, which eventually coalesced to form the present great island. Lanao was originally an elevated coral reef on which has been superimposed lava and volcanic ash to a great depth. Lake Lanao was formed when a lava flow dammed up a deep ravine or mountain cañon.

The fish fauna of Lake Lanao comprises one eel (*Anguilla celebesensis*), one species of *Ophicephalus* (*Ophicephalus striatus*), and seventeen or more species of Cyprinidae. The young eels make their way up from the sea to the lake, apparently through underground channels impassable to other fishes. Such channels can be seen at the Maria Cristina Falls, pouring water out through the side of the cañon below the falls, and at Lake Nunungan where the outlet of the lake disappears under a mountain, emerging a five hours' march distant. *Ophicephalus striatus* was introduced by man a long, long time ago, just as the Malays have done all over Malaysia in both historic and prehistoric time.

The seventeen species of Cyprinidae thus far described by me from Lake Lanao have all descended from a single parent stock and therefore present a very interesting problem in evolution. It is the purpose of this paper to present a tentative arrangement of the relationships of the Cyprinidae of Lake Lanao, and incidentally

of all the Philippine Cyprinidae belonging to the genus *Barbodes*, and also to call attention to the remarkable opportunity the fishes of Lanao present to the student of evolution who wishes to investigate a large scale experiment now being conducted in one of the recesses of the laboratory of Dame Nature.

First, it is necessary to consider all the Philippine members of *Barbodes* occurring outside Lanao, since they are closely related to one another and to the Lanao species.

Barbodes binotatus Cuv. and Val. is unquestionably the ancestor of all the Philippine species of Cyprinidae here considered. It is a fish of very wide distribution in the Malayan Archipelago, occurring from Sumatra and Singapore to Lombok and Borneo, and on into the southern Philippines. In the ancient land bridge now called the Sulu Archipelago it found a home in Tawi Tawi and Basilan and reached Mindanao, spreading over the whole of the latter island with the exception of the isolated plateau of Lanao.

Barbodes binotatus occurs in Mindanao from sea-level to an altitude of over a thousand meters in Bukidnon. It is a powerful swimmer, able to ascend mountain torrents, but is prevented from reaching the Lanao plateau by impassable waterfalls.

In the days when Sunda Land occupied a large part of what is now the South China Sea and Borneo and Sumatra were the outlying fringes of this great body of land, Palawan and the Calamianes were a part of what we now call Borneo. By this land bridge *Barbodes binotatus* migrated northward along the eastern shore of the China Sea. Later on when Sunda Land sank and the northern spur of land narrowed greatly and became the detached islands of Balambangan, Balabac, Palawan, Culion, Busuanga and Mindoro, the parent stock of *Barbodes binotatus* was cut off and isolation was free to work.

On Palawan, and perhaps elsewhere, *Barbodes binotatus* variety *palawanensis* became differentiated. From it in turn arose the following species:—(1) *Barbodes ivis* (Seale), occurring on the islands of Balabac, Palawan and Busuanga; (2) *Barbodes manguaoensis* (A. L. Day) and (3) *Barbodes bantolensis* (A. L. Day), both known only from Lake Manguao in northern Palawan; and (4) *Barbodes hemictenus* Jordan and Richardson, known only from Northern Mindoro.

In Mindanao with its heavy rainfall, great rivers, and great range *Barbodes binotatus* was undifferentiated over the greater part of the island. The peninsula of Zamboanga, without large streams and with little connection with the rest of the island, developed the closely related species *Barbodes quinquemaculatus* (Seale and Bean), thus far known only from the vicinity of the town of Zamboanga and the near-by island of Basilan. *Barbodes montanoi* (Sauvage) is another very closely related and doubtful species which may not be different from *B. binotatus*. It was obtained by the celebrated traveler Montano in 1880, from the Simulao River, an eastern tributary of the Agusan River, Mindanao, but has not been collected since although I looked for it in the Agusan Valley.

When the Lanao mountain ravine was dammed long ago it is self evident that the Cyprinid ancestor of the Lanao fishes of to-day was imprisoned in the headwaters of the stream. Gradually the mountain stream changed to a large lake with a great diversity of conditions, but of course there were always swift tributary mountain brooks and creeks, in which conditions would remain relatively uniform. Manifestly the changing conditions in the lake, with all its varied assortment of environments, imposed new demands upon the parent stock of fishes and eventually various new forms of more or less permanence or fixity appeared, some of which survived while others may have disappeared. To me, at least, it seems that this state of flux or transition for certain groups now living in the lake is still in existence.

It is not possible to say with absolute finality whether *Barbodes binotatus* or *Barbodes quinquemaculatus* dwelt in the fluvial system which was converted into Lake Lanao. The two species are very close, but a study of their distribution seems to indicate that a variety or rather geographical race of *B. binotatus* was the original inhabitant. I have previously indicated (*Philippine Journal of Science*, vol. 24, 1924, p. 291) that specimens from the coastal streams of Lanao, Misamis and Bukidnon differ in color and general form from those collected elsewhere.

With the formation of the lake and the isolation of the entire plateau, *Barbodes binotatus* disappeared and in its stead appeared *Barbodes tumba* Herre. This well-marked species occurs in mountain streams and lakes all over the Lanao plateau, with the single exception (whether real or apparent is not yet determined) of Lake Lanao itself. I have obtained it from all the other lakes of the plateau, from tributaries of the great lake as far up as fish can ascend, and in the boiling waters of the Agus, outlet of Lake Lanao.

As Lake Lanao attained maturity, various offshoots from *Barbodes tumba* arose, each with special adaptations for a particular environment.

One set of species took up life in the clear blue surface water about the great forests of *Potamogeton* lying offshore in depths of 5 to 15 meters. Here they fed, reproduced and swam about in great schools in the open spaces well offshore. A study of thousands of specimens seems to show the following named species to form a direct line of succession from *Barbodes tumba*, all living under the conditions outlined above: *Barbodes amara* Herre; *Barbodes lanaoensis* Herre; *Barbodes lindog* Herre; *Barbodes disa* Herre. *Barbodes lindog* is the culmination of this series, and from it arose *B. disa* on the one hand, and on the other the singular *Spratellicypris palata* (Herre).

Barbodes tumba has also given rise directly to at least one species which has become habituated to life at a

greater depth than the other Cyprinidae of Lake Lanao. Consequently it is rarely seen except after great storms at the height of the rainy season, or in midwinter. When the waves are rolling unusually high it approaches the surface and may be caught in the little gill nets used by all Maranao fishermen to catch Cyprinidae from the lake. This deep water inhabitant is *Barbodes baoulan* Herre, the most highly prized food fish of the Lanao plateau.

In shallow muddy bays, where the water is a mat of vegetation, a real lacustrine jungle, several other species have developed. The species living in such places are usually blackish or black, or dusky bronze. It is very noticeable that life in such a habitat causes most species of fish to be highly melanistic. The swampy lowland lakes of Cotabato Province, Mindanao, afford excellent illustrations of this. *Glossogobius giurus*, which well deserves its Tagalog name of *Bia ng puti* (usually written *Biang puti*) or "White goby," develops such marked melanism in Lake Buluan and the adjacent Liguasan Marsh that its blackened condition makes it difficult to recognize at a casual glance.

Another line of evolution from *B. tumba* has given us the pot-bellied little *Barbodes sirang* Herre, which is often entirely black in life. The maximum size of this species, 60 mm., is half or two thirds that of the next smallest Cyprinidae of Lake Lanao. I discovered *B. sirang* on my last visit to the lake, in July, 1931, and know nothing of its habits.

A line of development entirely distinct from those already mentioned has given us *Barbodes flavifuscus* Herre, from which has arisen *Barbodes katolo* Herre. With their very large, clumsy looking heads these two species are markedly different from all those previously named. The evidence is not conclusive, but I believe that *Barbodes flavifuscus* is an offshoot from *Barbodes amara*. Certainly I can not place it elsewhere with our present knowledge.

From *Barbodes katolo* have come two closely related genera, *Cephalokompsus* Herre and *Ospatulus* Herre. *Cephalokompsus* is distinguished by the possession of a continuous postlabial groove on the lower jaw, which is not interrupted beneath the chin as in *Barbodes* and most genera of Cyprinidae. The only species is *Cephalokompsus pachycheilus* Herre.

Ospatulus is separated from other genera by its truncate mandible, which is much shorter than the upper jaw. There are two species, *O. truncatulus* Herre and *O. palaemophagus* Herre.

Barbodes clemensi Herre is a large, thick, robust fish which occurs in the lakes of the Lanao plateau, particularly in Lake Lanao, where it, too, sports about in the groves of *Potamogeton* along with *Barbodes lanaoensis* and *Barbodes lindog*. It is a handsome fish, dark green above and yellow beneath, with a golden or brassy luster on its sides. Its size and color at once distinguish it from the various fishes whose lines of descent have already been traced. Adult specimens will average 175 to 200 mm., standard length.

Associated with *B. clemensi* by its color and general appearance, and attaining a still larger size so that it is easily the largest of Philippine Cyprinidae, is *Barbodes manalak* Herre, which occurs only in Lake Lanao, as far as yet known. Adult specimens measure from 190 to more than 250 mm., standard length, and are very bulky for their length.

There is a strong probability that *Barbodes clemensi* and *Barbodes manalak* arose directly from *Barbodes binotatus*, but it is also possible that they developed from *Barbodes tumba* without any connection whatever with the lines culminating in *B. lindog* or *B. katolo*.

The most remarkable of all the Lanao Cyprinidae has taken to life in the boiling eddies and swirling mad torrent of the Agus, the outlet of Lake Lanao. This fish is *Mandibularca resinus* Herre, a genus and species set apart from all other Cyprinidae by the development of

the mandible. The lower jaw has become an extraordinary elongated, upward curved, spatula shaped, fleshless organ. It is so strange that Dr. David Starr Jordan said it might almost be made the type of a new family. This species, which is fully as large as *Barbodes clemensi*, probably occurs also in Lake Lanao, but thus far I have only seen specimens from the rapids and whirlpools of the Agus, some caught by myself and some by Marinao anglers.

The origin of *Mandibularca resinus* is not very difficult to trace, as now and then one finds individuals of *Barbodes clemensi* in which the lower jaw suggests the singular development so characteristic of *Mandibularca*.

Barbodes tras Herre is a peculiar Cyprinid which apparently is not very closely related to any of the other species thus far known from the lake, yet its structure suggests that it too is an offshoot from *Barbodes flavifuscus*. However this may be, it has evolved in a way entirely distinct from any of the others mentioned.

While working out the above-named forms, I have encountered many anomalous individuals which can not be placed with any certainty. Some are apparently hybrids, others seem to be valid species but more and better material is needed to determine their status. Some individuals, especially those belonging to the highly melanistic *katolo* group, seem to be in a state of flux with a comingling of the characters of several species.

I feel that the Cyprinidae of Lake Lanao present to the zoologist, within a limited and sharply isolated area, a miniature illustration of what certain Cladonias present on a world-wide scale.

From the outline given it can be seen that the fishes of the Lanao plateau present a highly interesting problem to the student of evolution, one which would well repay an intensive study. Something has been presented here from the unfashionable view-point of the field naturalist, in the hope that some modern technician might take up the study of the Lanao Cyprinidae.

It would seem that here we have an experiment being conducted before our eyes on a scale unapproachable by man. Let one of the "new" biologists leave his laboratory and apply his methods to the fishes of Lanao; perhaps he might then make a real contribution to the study of evolution. By spending six months on the shores of the lake he could obtain with great ease all the material he could handle, as the Marinao fishermen bring in thousands of fish on market day, often many canoe loads of each of the commoner species.

By studying several thousand fresh specimens of each of the ten most abundant species, and studying all the specimens obtainable of the rarer species and all the anomalous individuals, he could do much toward unraveling the phylogeny of the more puzzling forms and could perhaps place in their proper sequence the doubtful cases and those forms which seem to be examples of hybridism.

With the foundation indicated, his statistical analysis of species would have real value and would throw light upon the evolution of so many species from one parent species.

MUTATION AND ADAPTATION AS COMPONENT PARTS OF A UNIVERSAL PRINCIPLE:

I. THE PRINCIPLE OF RHYTHM OR PERIODICITY

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ALL conscious knowledge is fragmentary. The learned and unlearned know this fully. It is only the half-learned, the superficially educated, that manifest impatience when such an idea is expressed. To the former it is no surprise to see a familiar idea reappear in a clearer but still fragmentary form, or to hear something that they have known intuitively expressed in inadequate words. To the former the ideas expressed in this short series of papers will be familiar and beautiful. They are therefore fragments written for the learned and the unlearned. Only these will be able to appreciate how much more the writer might have said, had he known how to say it. These alone will realize how much more fruitful this group of essays would be if they could have been written one hundred years or even fifty years hence. They alone will be able to hear the faint voices of the instruments of a great symphony of which the score could not be recorded here. These essays therefore become autobiographical in a certain sense. This seems to be the best and simplest way to approach the problem of saying something about the vague but intensely interesting notions that have grown up in the writer's mind.

For more than twenty-five years the writer has been more or less continuously perplexed by a certain lack of harmony and unity in our views of nature. It seemed that in the various fields of science and their subdivisions many things were being seen but partially and others were seen as through a thick haze. There seemed to be some relationship between the hazy views and the partial views, but one could not lay his fingers on it in a tangible

form. One day about seven years ago a number of these views came into juxtaposition so that the writer could "sight through" them, so to speak. In a few hours much of the perplexity was clarified. In the interval since then the writer has tried a number of times to write out a view of the things seen. This has proved to be a most difficult task.

With this word of apology and explanation we may proceed with the exposition of what appear to be fragments of a new universal principle.

In our conventionalized interpretation of physical and chemical processes we habitually stay within certain boundaries. If we step out of these boundaries we either lose our bearings completely or else we find ourselves seeing familiar things in a new way. In this essay and in those to follow we are certainly stepping out of boundaries, but we believe that we have not lost our bearings and that we are seeing things in a new way.

Some years ago the writer sat down to carefully consider what happens when we slowly warm a suitable mixture of oxygen and hydrogen under a piston head of constant weight. As every one knows, if we plot the temperature of the gas against its volume, we obtain a straight line and when we have just about convinced a novice that we can fully describe the behavior of this system, by saying that the volume of the mixture is a linear function of the temperature, an explosion occurs. If the piston is still in place and the surrounding temperature is still above 100° C. the volume of the vapor in the cylinder is almost exactly two thirds of what it was before the explosion. If we now continue heating the water vapor present in our apparatus we again obtain a linear volume-temperature curve having the same slope as the other. We may heat or cool our gas mixture or our water vapor and the volume will increase or decrease in accordance with this simple linear law. We have then two completely reversible states connected by a sharp discontinuity representing a radical change that is not

readily reversible. Or if we were to consider it in descriptive terms, we have two chemical species, having similar properties under these conditions, connected by a discontinuity, which represents the transformation of the one into the other. Obviously, these two kinds of change are widely different processes.

Such situations, in which we have two steady states connected by a sharp change of some sort, are very abundant in chemistry and physics, and it is obvious that they are related in some way if we could express the relation in proper terms. The first seemed to be identical with the principle of adaptation, while the other strangely enough appears to be the principle of mutation. That adaptation is probably a universal principle has been pretty clearly recognized in one way or another for a long time. That mutation is also a universal principle and that it involves a type of change that is distinctly different from that usually understood by adaptation has not been so clearly perceived. Moreover, that these two principles are related to each other, chronologically at least, has been realized by many. Many of us know that when things are moving smoothly, *i.e.*, situations are adaptable, cataclysmic changes sometimes break in. A tornado may appear on a mild summer day; an earthquake may take place in the middle of the night. But here the two principles exist merely in juxtaposition. In some other instances the relation is seen to be closer and more rational, but the idea that these two principles are really closely and integrally related and probably constitute parts of a single universal principle has perhaps never been clearly stated before.

Since it is the purpose of this essay to attempt to establish not only a causal and genetic relationship between the two principles, when viewed separately, but also to establish as fully as possible the idea that they constitute parts of another more comprehensive universal principle in which both are combined, it will be wise to review

briefly but as clearly as possible existing knowledge on the two component principles.

ADAPTATION

Only a few great principles are known which have a definitely recognized universality. Among these are the law of conservation of energy and the law of conservation of matter. Bancroft¹ some years ago pointed out that the Le Chatelier-Braun principle or the "principle of mobile equilibrium" of chemistry is also a universal law. In Bancroft's words: "The physicists call it the Theorem of De Maupertius or the Principle of Least Action. By the biologists it is known as the Law of the Survival of the Fittest, while the business man speaks of the Law of Supply and Demand. The broadest definition of it is that a system tends to change so as to minimize an external disturbance."

This restatement of Le Chatelier's principle is certainly not an improvement upon the statement of it made by its author, for the field in which he applied it, but the broader statement permits of testing its applicability over wider ranges. Considered in this way, it is rather obvious that in each department of thought the principle has been rediscovered in some form and a useful name invented. If we wished to give a comprehensive and at the same time a suggestive name to this principle it could perhaps best be called the adaptation principle. Bancroft gathered together a great many scattered examples, from biology, of the apparent action of this principle in an effort to show that it really is a universal principle. However, this problem of tracing the influence of environmental factors in living organisms is much more difficult than it is in the case of non-living systems. But even in the non-living systems it is successful only with the simplest systems. Colloidal and complex systems develop mystifying difficulties, when considered in this way. It was for this reason that we fixed our attention

¹ *Jour. Am. Chem. Soc.*, 33: 91-120, 1911; *Science*, 33: 169-79, 1911.

on the simple mixtures of hydrogen and oxygen or on our water vapor, as was stated above, and perhaps we had better return to such examples again.

We have just spoken of tracing the influence of environmental factors in the case of simple non-living systems as being relatively simple. This idea requires some elaboration on the basis of familiar facts. When we heat an ordinary liquid, such as benzene, there is a slow change of temperature. If we continue heating the liquid we presently reach a temperature, technically known as the boiling point, at which there is an enormous increase in volume without any measurable change in temperature. This continues as long as heat is supplied or until the benzene is all vaporized. If we now keep this benzene at its boiling point, or a little above, we can heat it and cool it and it behaves like a true gas. The line representing its adaptation to temperature resembles the line for this change in the liquid state, except that it has a somewhat greater slope. The lines representing these

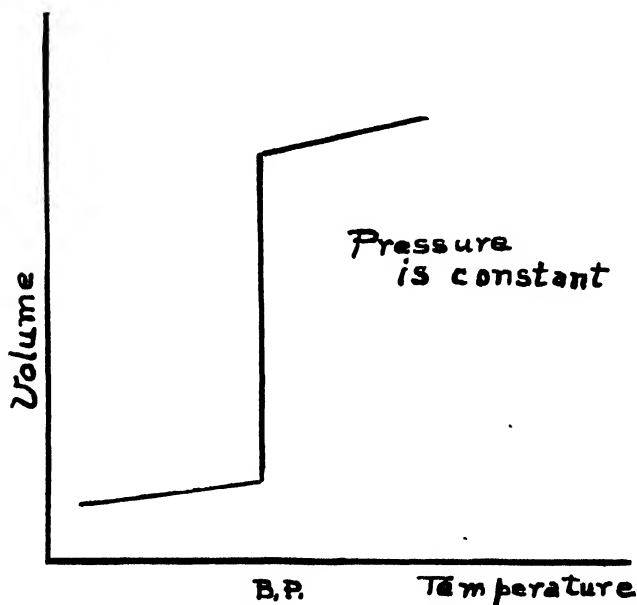


Fig. 1.

two adaptation curves may be connected by a vertical line (Fig. 1) that represents the sudden mutation of benzene from the liquid to the vapor state and *vice versa*. Below and above the boiling point the substance obeys Le Chatelier's Principle; its behavior at the boiling point is anomalous from this standpoint.

This break has been the subject of much study in the case of gases that are more difficult to liquefy and is now pretty well understood.

In 1869 T. Andrews found that if carbon dioxide be gradually compressed in a vessel suitable for the observation, the volume diminishes more rapidly than would occur if Boyle's Law correctly described the behavior of the gas; and when the pressure attains a certain value, the gas begins to liquefy. A further decrease in the volume does not change the pressure, but only increases the quantity of gas liquefied. At length when all the gas has liquefied, a large increase of pressure causes only a minute decrease in the volume of the liquid, since liquids in general undergo but a small change of volume on compression.

If the experiment be made with carbon dioxide at 0°, the gas commences to liquefy when the pressure has attained 35.4 atmospheres; if at 13.1°, liquefaction commences at 48.9 atmospheres pressure; while if the temperature exceeds 31°, no pressure, however great, will liquefy the gas. Other gases exhibit similar phenomena. For each gas there is a particular temperature above which liquefaction is impossible; Andrews called this the critical temperature of the gas.²

Knowledge concerning this phenomenon has been greatly increased since 1869 and it has now long been customary to represent this phenomenon of critical temperature, in the pressure-volume diagram of a gas, by a zone bounded by a dotted line (Fig. 2), in which the gas is in the transition state from gas to liquid or *vice versa*. This is the conventional way of admitting and symbolizing the fact that the adaptation principle or Le Chatelier's principle does not hold while the gas or vapor is in this zone. The zone outside the dotted line is a zone of gradual change in response to changes in pressure or volume in relation to temperature. The zone inside the dotted line is a zone of the liquid state. The dotted line is the zone of sudden transition between the liquid and

² J. W. Mellor, "Modern Inorganic Chemistry." 1st ed., p. 679, 1922.

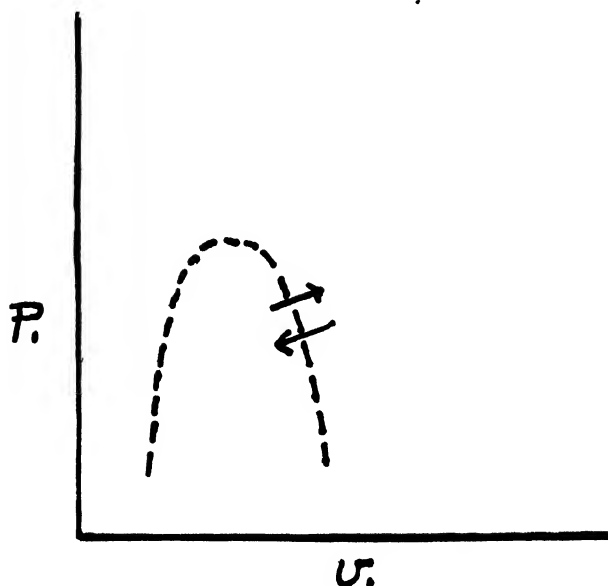


Fig. 2.

gaseous state. In order to predict the behavior of a substance with reference to this diagram it is necessary to know the boundaries of the dotted area. That is, in biological terms we must know the "life-history" of the substance.

In all the preceding we have merely reviewed a few well-known facts of elementary chemistry. The only novelty introduced is that we have used some biological terms in the course of the discussion. When it was found that these biological terms could be used in this way, it was suddenly realized that these breaks in the volume-temperature diagrams might correspond to the phenomena of mutation in living organisms, discovered by DeVries and announced in 1900. It was evident at once that, if such a relationship could be shown to exist, mutation and adaptation would necessarily have to be regarded as universal principles, and that they may therefore be definitely integrated in some way with or through the life-history of the system under considera-

tion. Before proceeding to investigate this suggestion it seemed wise to review the work of DeVries on mutation to refresh our memories of what he did and what he learned from it. We shall attempt to see his work in its own historical setting and shall use his own words as largely as possible.

VARIABILITY IN PLANTS

Lamarck founded the theory of a common descent for all living things. But his evidence was scanty and failed to convince. About a half century later Darwin collected so much evidence as to overwhelm the opposition. The broad proofs of the theory of evolution presented by him are independent of the means by which new species are produced. This question of the origin of species, however, appeals directly to the imagination, and Darwin collected all the evidence that he could find. The rapid victory gained by his views was mainly due to his discussion of this minor point. From a study of available data he concluded that the principle of natural selection guides evolution. The theory of common descent is Darwin's theory and remains unshaken, even if our conception concerning the mode of descent and the importance assigned to natural selection by Darwin requires some revision on the basis of new knowledge of variability acquired since Darwin published his results. Although the evidence was so incomplete, Darwin felt it necessary to make a decision of some kind as to the guiding principle of evolution and designated natural selection. It was thus universally assumed that the changes by which species originate are slow, almost invisible and may accumulate, in the lapse of time, to any degree. All the characters of living organisms were simply assumed to be due to this slow process of gradual evolution guided by natural selection.³

Shortly after the publication of Darwin's *Origin of Species*, the Belgian Anthropologist, Quetelet, submitted the variability in dimensions of the

³ The above is the author's abstract of pp. 1-5 of "Plant Breeding" (1907), by H. DeVries.

different parts of the human body to a statistical investigation. He discovered that this kind of variability follows distinct laws and that these laws agree, in the main with the law of probability. Small divergencies from the average are numerous, larger discrepancies are rare, and the larger the rarer they are. Variability is thereby limited, and is subject to a return to the average condition. It may be moved from this average, to some extent, by a change in the outward conditions, or by a repeated selection in one direction; but as soon as these causes and this selection cease to work, a return to the average is unavoidable. Variability may augment or diminish qualities; it is linear, consisting of changes along a simple line, some being positive and others negative, but it does not strike out into new directions. It is no source of new qualities. The phenomena which are bound to such narrow limits can not be a basis for the explanation of the origin of species. It governs quantities and degrees of qualities, but not the qualities themselves. Species, however, are not in the main distinguished from their allies by quantities or by degrees—their very qualities may differ. . . .

From this discussion it may be seen that the slow and gradual changes of ordinary variability and the production of new characters are not of the same order. Variability, in the ordinary sense of the word, is a broad conception. It must be subdivided for the purpose of scientific investigation. The phenomena that follow Quetelet's Law are now considered as one group, which is called fluctuating variability or fluctuation, since the individual qualities fluctuate around their average. The processes by which new qualities are produced must be studied separately. Under the assumption that these processes are neither slow nor invisible but consist in leaps and jumps such as are popularly indicated by the name of sports, they are now called mutations, and this great subdivision of phenomena of variability is designated, in consequence thereof, as mutability. . . .⁴

. . . we may sketch the origin of species, according to the theory of mutation, in the following manner. Species are derived from other species by means of sudden small changes which, in some instances, may be scarcely perceptible, to the inexperienced eye. From their first appearance they are uniform and constant, when propagated by seed; they are not connected with the parent species by intermediates and have no period of slow development before they reach the full display of their characters. They do not always arise, but only from time to time. . . . Even as the new species are produced locally and as the effect of local causes, the struggle for life and natural selection decide concerning the survival according to the local conditions.⁵

DeVries (*loc. cit.*, p. 11) describes some of his experiments on mutation as follows:

The double variety of corn-marigold (*Chrysanthemum segetum*) arose in my garden in a culture in which I was increasing the number of the ray florets by continuous selection. During four years I had succeeded in in-

⁴ DeVries, *loc. cit.*, pp. 5-6.

⁵ *Ibid.*, pp. 9-10.

creasing this number to about sixty on each head, starting from the cultivated variety with an average of twenty-one. All the ray florets, however, belonged to the outer rows of the head, as in the original variety. At once a plant arose which produced some few ligulate florets in the midst of the disc. This indicated the production of a double race. When the seeds of this mutating individual were sown, the next year, they yielded a uniformly double group; and from this time the new variety remained constant.⁶

In another volume DeVries summarizes his views as follows:

Summing up the results of this very hasty survey, we may assert that species remain unchanged for indefinite periods, while at times they are in the alterative condition. Then at once they produce new forms often in large numbers giving rise to swarms of subspecies. All facts point to the conclusion that these periods of stability and mutability alternate more or less regularly with one another. Of course a direct proof of this view cannot, as yet, be given but the conclusion is forced upon us by a consideration of known facts bearing on the principle of constancy and evolution.⁷

The above constitutes a suggestive review of the relation thought to exist between the principle of adaptation or fluctuating variation, to use DeVries' term, and the principle of mutation in plants as first described by him. That is, there are long periods of adaptation with short periods of mutation interspersed between. So far the behavior of species of plants resembles that of the behavior of the species of matter discussed above. The question naturally arises as to whether this similarity is not merely coincidence. The idea is certainly not familiar and is to say the least disturbing. It is because of this disturbing quality that it seems wise to discuss the idea in some detail in terms of more or less familiar phenomena. And it will be our purpose in the remaining parts of this essay to do just this, in order to see if we can grow familiar with the idea.

Some one will suggest that when the history of botanical species is given in this way or the relations of vapors and liquids is considered in the terms given above, it

⁶ The details of this are given in "The Mutation Theory," H. DeVries, Vol. II, pp. 161-94, 1910.

⁷ H. DeVries: "Species and Varieties; Their Origin by Mutation," p.

sounds like an abstruse description of the stock market, which moves along steadily with prices fluctuating a little in accordance with the law of supply and demand, until another variable influences the market and then it goes wild, *i.e.*, mutates for a while. When the tension has quieted down, or the explosion has taken place, whatever is left behaves again according to the law of supply and demand. In other words, business is like benzene. It will adapt itself to changes within a certain range according to the law of give and take, or the adaptive principle, but after a while a limit is reached. In the case of business as well as benzene a reorganization then takes place. In case of business the capitalization may be decreased or increased to a marked extent without cessation of business activity; in the case of benzene heat is given off or absorbed in considerable amounts without an accompanying change in temperature. The analogy is close and striking. The history of a given business like that of benzene is usually a long period of fluctuating variations or adaptations to small changes, interspersed with short periods of mutation, in which changes resembling those associated with critical temperature and pressure in a gas take place.

THE PRINCIPLE OF RHYTHM

A close view of the possible interrelation of the principle of adaptation and of mutation in organisms, against a physico-chemical background of the behavior of gases and liquids, gives one a distinct shock. It appears at first that discontinuity is a basic principle of the universe and thus reduces us to incoherence because it appears to provide a method by which natural phenomena may escape unified mathematical treatment. But here again we may extend our view, and presently we see that the adaptation and mutation portions of our curves may be considered as constituting a single harmonic cycle. These apparently antipodal propositions may be viewed as parts of a continuous curve. We can view the two

separate processes as a unit and bring the separate universal principles under the head of another universal principle, which we may name suggestively as the principle of rhythm or harmonics.

Considered in this form, as a rhythm, the two separate principles seem to develop a fundamental quality of familiarity. They seem less remote from our common experience when considered together as a rhythm than when viewed as separate detached principles. In fact, we seem to have a physiological sense that recognizes rhythm as the most fundamental principle in the universe. Moreover, it is comforting to know that this principle of rhythm or harmonics is already amenable to systematic mathematical treatment, whereas a succession of discontinuities, or a systematically unrelated series of adaptations and mutations, is not amenable to orderly unified treatment. It is also well known that heat, light and electricity are transported through space as rhythmic or harmonic pulsations. In fact, our present knowledge of harmonic functions was mainly achieved in the study of electricity and sound, and the modern development of the radio arose in part from a recognition of the fundamental similarity and reciprocal transformability of these two forms of energy.

The periodic revolution of the earth about the sun and the inclination of its orbit determine the rhythmical seasonal variations of the year. The periodic rotation of the earth before the sun determines the rhythm of day and night. These well-known facts fundamentally condition and give rise to a series of periodic rhythms in the life and vital activities of all plants and animals in which periods of rest and dormancy alternate with periods of activity.

When we consider animals and plants more closely it is evident that rhythm is built into the constitution and functions of the organisms. Thus the beat of the heart, peristalsis in the gastrointestinal tract and the act of respiration are familiar physiological rhythms upon

which life depends. These rhythms are regulated by an involuntary mechanism. The functioning of a voluntary muscle is known to be controlled by a cyclic process of action and restoration, which resembles these rhythmic or periodic processes under discussion. The rhythmical banding of bones is another interesting instance in physiology, which although it is a purely physico-chemical process is not fully understood. In fact, quite a few chemical reactions are known which manifest a rhythmical periodicity, but the subject has not been given the attention which it probably deserves.⁸

When we come to consider the matter fundamentally we must see that all such rhythms as those that we have considered are dependent directly or indirectly upon the sun. All space within the solar system is filled with energy that is moving in radiating straight lines from the sun. This energy field has the property of existing in a marvelous state of constant pulsation and of being constituted of a large assortment of wave-lengths or frequencies. We now know that this rhythmical discharge of the sun is impressed upon or implanted within everything in its path. Plants have the most efficient "receiving sets" for this energy, but use and convert only a small fraction of that which falls on the earth. It is believed that these pulsations emitted by the sun are in turn due to the transformations of atoms and molecules in the sun. Such an interpretation is justified by recent developments in the study of the atom. But whatever the ultimate source of the energy may be, there is no possible doubt as to the rhythmical quality of the radiations and the fact that this quality is present in every system that is acted upon by them.

For a moment it seemed that the familiar force of gravitation or steady pull does not give rise to rhythms, but it becomes quickly clear that this is untrue. The oceanic tides represent a most spectacular gravitational

⁸ Cf. Hedges and Meyers: "The Problem of Physico-Chemical Periodicity." Longmans, Green and Company.

rhythm. The rhythmical muscular activities and path of motion involved in walking, running, hopping, flying and other forms of locomotion are all due to the action of gravitation. The eccentricities of planetary orbits and the fact that more than one body is revolving about our sun in the gravitational field gives rise to harmonic variations in the actual path that would be traversed if but one body was involved. This is true in the infinitely large scale of planetary motion. The same appears to be true in the realm of the infinitely small scale of electronic motion within the atom. If a planet were expelled from our solar system or a new one were shot into it, a period of adaptation would necessarily follow such a "mutation," during which the bodies involved would work out a new system of harmonic variations in response to the disturbance and come to dynamic equilibrium. This is also apparently true of the electronic systems in atoms and is a subject of very active interest and investigation at the present time.

RÉSUMÉ

In the above we have shown that when considered in a certain way there is a striking similarity in the behavior of vapors and liquids on both sides of the critical zone, in the behavior of a reactive gaseous mixture on both sides of the reaction zone and in the behavior of botanical species on both sides of what might be called the zone of mutation. We have in all these cases two continuous states connected by a discontinuous state. We found that in these cases the life history of the species, whether it be matter or a living organism, must be known in order that its future behavior may be predicted. From familiar data submitted it became evident that such apparent discontinuities were due to the influence of a new variable; that when the system under consideration has complied with the requirements of this new variable it is found to have been reorganized in some way, and in its new state it again becomes amenable to the law of adap-

tation. Such apparent discontinuities, when they are found to occur with regularity, are classed as periodic or harmonic processes. The fact that such rhythmic changes should be expected is established by reviewing the universal occurrence of such phenomena even in totally unexpected places.

This periodicity in part arises from the periodic relations of the earth to the sun and in turn in part from the impressed periodic quality of all forms of energy in the state of transmission from the sun and of reception by the earth. The earth and everything on it lies in this complex pulsating field of energy. There is therefore no reason to doubt the universality of rhythm or periodicity. It remains to be determined whether in the subsequent parts of this essay, the interrelations of these and related phenomena can be developed clearly enough to pass muster as the qualitative statement of a universal scientific principle.

SHORTER ARTICLES AND DISCUSSION

CONSTITUTIONAL EFFECTS OF THE HAIRLESS GENE IN DIPLOID AND TRIPLOID DROSOPHILA¹

THE Hairless gene of *Drosophila* in heterozygous condition deletes bristles from the head, thorax and abdomen and shortens the veins of the wings. The Hairless gene in homozygous condition kills the developing fly embryo before it can emerge as an imago. Why should these individuals die? Certainly the few bristles removed in the heterozygous fly do not suggest that Hairless should be death dealing when homozygous. In double dose this gene has apparently extended its physiological action. We are left to guess the steps by which this extension of reaction manifests itself. The student of the developmental action of the gene needs to know what these steps are, since this knowledge would possibly indicate the mechanisms involved in the whole process of gene action.

Triploids offer a possibility of making these observations since they furnish intermediate grades in the balance between normal wild type and Hairless genes. A wild type diploid has two normal genes, $++$, giving a gene ratio of 2:0. A heterozygote, $+H$, has a ratio of 1:1. A homozygote, HH , would have a ratio of 0:2. Triploids have three of these allelomorphs. A wild type triploid has the composition $+++$, a ratio of 3:0. A triploid with two wild type factors to one Hairless, $++H$, has a ratio of 2:1. A triploid with one wild type gene to two Hairless, $+HH$, has a genic balance of 1:2. A homozygous Hairless triploid, HHH , would have the ratio 0:3. Triploids and diploids thus furnish four distinct steps in genic balance, wild type, $++H$, $+H$, and $+HH$. Two more extreme possibilities, HH and HHH , do not survive.

Triploids with one Hairless gene, $++H$, seem to live and breed as well as wild type triploids, $+++$. Triploids with two Hairless genes, $+HH$, are markedly less viable than normal triploids. Of the few adults which survive, few breed, and those have few progeny. The appearance of the diploid and triploid types are shown in Fig. 1.

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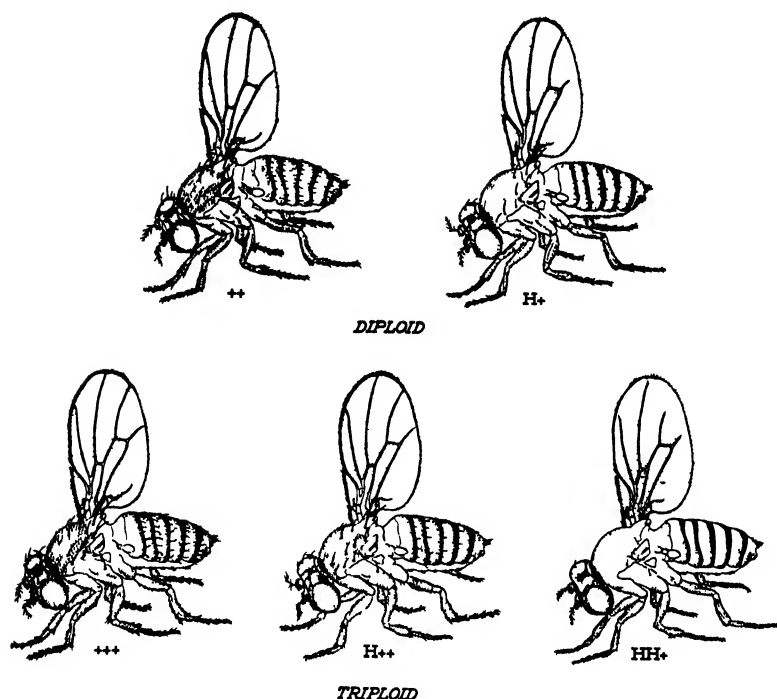


FIG. 1. Constitutional changes produced by the Hairless gene in diploid and triploid flies. The writer is indebted to Dr. Ralph G. Schott for making these drawings.

The wild type diploid, with bristles and wing venation typical of the species, is represented as the ++ type. The heterozygous Hairless fly lacks the two post verticals, the anterior dorsocentrals and most of the abdominal bristles. The fifth long vein fails to reach the margin of the wing and the fourth is thinned as it approaches the wing edge.

The triploid wild type is like the diploid save that the bristles are heavier. The triploid with one Hairless gene, ++ H, is intermediate between the wild type diploid ++ and the heterozygous Hairless diploid + H. It may lack one and sometimes both of the post-vertical bristles. Generally the anterior dorsocentrals are present. The fifth wing vein generally lacks but little of reaching the margin of the wing. The abdominal bristles are somewhat reduced in number. A triploid having two Hairless genes, + H H, is more extreme than any other type, clearly going beyond the heterozygous diploid in the expression of the effects of the Hairless gene. Most of the bristles on the

head, thorax and abdomen are absent, except that their basal rings remain. The fourth and fifth veins are sharply cut before they reach the wing margin. Often the second and sometimes the third wing veins fail to reach the margin.

Triploids furnish an intermediate step between the wild type and the heterozygous Hairless diploids. They also give a form beyond the heterozygous Hairless diploid. The reaction of the host to its gene content advances by steps determined by the balance between the wild type factor, +, and the Hairless factor, H. In each step the effects of the Hairless factor have been extended to other structures than those which the lower genic balance affected. The graded nature of the series gives the basis for the hypothesis that when the balance becomes greatly altered the developmental effects are extended still further to eliminate or lethally alter vital structures.

Mangelsdorf and Fraps² have shown a similar graded effect of the factor for vitamin A content of corn endosperm. The endosperm cells are triploid and may be white, y y y, pale yellow, y y Y, medium yellow, y Y Y, or dark yellow, Y Y Y, in their appearance and genetic constitution. The step alteration in balance brought about by the addition of one yellow gene, Y, and the consequent subtraction of one white gene, y, results in a uniform unit increase of the vitamin A content of the grain. It is impossible to say what the quantitative effect of removing a bristle may be, but qualitatively the different grades of hairlessness produced by different ratios of the allelomorphs H and + in the Hairless locus are certainly in accord with the view that these genes act like those in the yellow locus of corn.

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THE RELATION OF SULPHYDRYL CONCENTRATION TO SIZE INHERITANCE IN THE RABBIT

ALTHOUGH investigators have been trying for many years to solve the problem of the inheritance of size in animals, no one has as yet reached a solution which is completely satisfactory. However, we believe we have discovered a new function of one

² P. C. Mangelsdorf and G. S. Fraps, 1931. "A Direct Quantitative Relationship between Vitamin A in Corn and the Number of Genes for Yellow Pigmentation," *Science*, 73: 241-242.

of the biochemical substances, which plays an important part in tissue respiration. We are convinced that this new function is concerned with the phenomenon of size inheritance and the limitation of body size in the rabbit.

Professor Castle (1929¹ and 1931²) working with the rabbit, crossed a large (giant) race with a small (dwarf) race. The F₁ hybrids, which were intermediate in size, were then back-crossed to both the small race parents and the large race parents. In these experiments Castle had four chromosomes marked by genes which affect color of fur, but he did not find a gene which had an appreciable effect on size segregating with any of these marked chromosomes.

After a comparative study of twelve-day embryos from the same large, small and F₁ hybrid races mentioned above, Painter³ came to the conclusion that the size of the cell is the same for all races of rabbits, regardless of adult weight, and that the difference in cell number is responsible for the difference in adult weight. After making a comparative study of rate of segmentation of large and small race zygotes, Castle and Gregory (1929⁴) found that the large race eggs segment at a faster rate than do small race eggs. They also found that this difference in rate of segmentation could be detected as early as 48 hours after copulation and that the difference in size became more manifest as the gestation period increased. From further comparative studies on the embryological basis of size inheritance in the rabbit, Gregory and Castle (1931⁵) found that differences in cell number between the eggs of the large races and the eggs of the small races could be detected as early as forty hours after copulation. The adult body size of the races and genetic combinations studied by Castle, furthermore, coincides with the mean rate at which the eggs of the respective races segment.

Hammett (1930⁶) showed that the sulphydryl group is a stimulus to growth by increase in cell number, and that this chemical group is concentrated in regions where growth by cell proliferation is actively taking place. Although sulphur is

¹ *Jour. Exp. Zool.*, 53: pp. 421-454.

² *Jour. Exp. Zool.*, 60: pp. 325-338.

³ *Jour. Exp. Zool.*, 50: pp. 441-453.

⁴ *Jour. Morph. and Physiol.*, 48: 1, pp. 81-103.

⁵ *Jour. Exp. Zool.*, 59: 2, pp. 199-211.

⁶ *Proc. Am. Phil. Soc.*, 69, pp. 217-223.

widely distributed in body tissues, and although a potential source of the sulphydryl group is present in cystine and cysteine, there is little evidence to show that these acids exist free in the tissue. The tripeptide, glutathione, is, however, widely distributed, and it is probably the principal substance which furnishes the free sulphydryl in the body tissues. However, Benedict, Newton and Behre (1926⁷) reported the isolation of a sulphur containing nitrogenous base from blood, and Newton, Benedict and Dakin (1926⁸) showed this to be identical with ergothioneine. This substance, like glutathione, has a sulphydryl group, which, according to Mason (1930⁹), reduces iodine. In this paper no attempt has been made to distinguish between these two sulphur compounds and the total sulphydryl concentration has been expressed in terms of glutathione.

Since the adult size of races of rabbits differing in weight is controlled by the rate and duration of cell proliferation, Gregory and Castle (1931) suggested that the genes which affect size may be of a sulphydryl nature.

This study is the result of an attempt to measure the difference of sulphydryl concentration in the tissues of the large, the small and the medium sized races of rabbits, as well as in certain hybrid combinations. The data presented in this preliminary report indicate that adult weight is definitely related to sulphydryl concentration in the total body tissues of newly born rabbits which have fasted forty-eight hours.

A large Flemish race and a small Polish race were used in this study. They were from the same stocks as those which Castle used in his recent studies on size inheritance, and which Castle and Gregory used in their investigations of the embryological basis of size inheritance. In addition to the large and small races mentioned above, New Zealand Red and Albino Angora races were used. These were intermediate in size between the large and small races. In making chemical determinations the individuals of all races and genetic combinations were treated as uniformly as possible, and as a result the differences in concentration of sulphydryl represent genetic differences and can not be attributed to the result of environmental agencies.

The method of procedure was to take newly born rabbits from their mother as soon after birth as possible and allow them to

⁷ *Jour. Biol. Chem.*, 67, p. 267.

⁸ *Science*, 64: 602.

⁹ *Jour. Biol. Chem.*, 86, p. 623.

fast for 48 hours before the analysis was made. Each animal was analyzed separately. The whole carcass was ground with quartz sand in a mortar and filtered through cheesecloth. The residue was again ground and extracted with cold water. Three extractions were found sufficient to remove most of the substance which reduces iodine. The combined extracts were deproteinized with tungstic acid and the filtrate titrated with N/100 iodine in the presence of potassium iodide, using starch indicator (Okuda¹⁰). The process was standardized with pure glutathione (Eastman).

The sulphydryl concentration of 90 rabbits of different races varying in adult size, analyzed on a 48-hour fasted basis, are summarized in the following analysis. Twenty-eight rabbits from the large Flemish race, which has an adult weight of over 5,000 grams, have a range of concentration of glutathione of 36 to 61 milligrams per hundred grams of body weight with a mean of $43.7 \pm .806$, standard deviation 5.5. The New Zealand Red rabbits, the females of which average 3,700 grams and the male 3,000 grams, have a range of glutathione concentration from 19 to 43 milligrams per hundred grams of body weight with a mean of $30.8 \pm .911$, standard deviation 6.04. On account of limited material only eight young of the small race, the adults of which have a body weight of approximately 1,600 grams, are included in this series of analyses. The range in concentration of glutathione of the eight small race individuals is from 24 to 32 milligrams per 100 grams of body weight with a mean concentration of 27 milligrams. Since the data for this group are limited, we should consider this figure as only a close approximation to the mean concentration until more sufficient numbers of analyses are obtained. There is a possibility that the curve for concentration of glutathione concentration may be bimodal in the small race. The newly born young from an Albino-Angora race, the adult weight of which is approximately 3,000 grams, are also included in the analysis. The sulphydryl concentration is 25 and 26 milligrams per hundred grams of body weight. On account of the normal variation in concentration within a race, it is impossible to determine the mean concentration of sulphydryl of the Albino-Angora race, from the analyses of these two specimens. However, the results of these two analyses are significant, since they fix the range of concentration in the lower limit of the scale.

¹⁰ *Jour. Biochem.* (Japan) 5, 201 (1925); 217 (1925).

The data obtained from the hybrids between the various races are interesting. The range of glutathione concentration of ten Angora-New Zealand Red hybrids was found to be from 23 to 41 milligrams per hundred grams of body weight, with a mean of 31.8. The mean concentration of glutathione for this hybrid series closely approximates that of the New Zealand Red parents.

The Flemish-Polish hybrids form a very interesting and important series. The concentration of glutathione ranges from 27 to 44 milligrams per hundred grams of body weight, with a mean of $34.7 \pm .59$, standard deviation 4.15. When the mean concentration of sulphhydryl of the Flemish-Polish hybrids is compared with the mean concentration for the Polish and Flemish parental races, we find that it is distinctly intermediate between the two. All the Flemish-Polish hybrid material was obtained by mating Flemish females with Polish males.

Flemish doe number 242.1 furnishes some very critical data. She was first mated to a Flemish male, and seven of the young which resulted were analyzed for sulphhydryl concentration. The range was from 43 to 61 milligrams of glutathione per hundred grams of body weight with a mean of 50.3. Flemish doe 242.1 was next mated to a Polish male, and the resulting litter of thirteen was analyzed for glutathione concentration. The range in concentration of sulphhydryl was from 27 to 44 milligrams per hundred grams of body weight with a mean concentration of 35.4. It is clearly evident that zygotes resulting from large race eggs, fertilized by small race spermatozoa, are lower in concentration of glutathione than pure large race zygotes. From these data, it is also evident that high concentration of glutathione is incompletely dominant to low concentration. This agrees with the evidence from breeding tests made by Castle (1929) in which he showed that Flemish-Polish hybrids are intermediate in size, the weight of the mature hybrid being approximately 3,500 grams. It also agrees with the embryological evidence put forth by Gregory and Castle (1931). They found that the rate of segmentation of hybrid eggs between the two races are intermediate when compared with the two parent races.

The mean concentration of glutathione, together with their differences for the different races where numbers are sufficient for statistical analysis, are summarized in the following table:

Race	Total	Mean concentration of glutathione
Flemish	28	$43.78 \pm .706$
Reds	20	$30.8 \pm .911$
Difference		12.98 ± 1.151
Flemish	28	$43.78 \pm .706$
Hybrids (Flemish \times Polish)	22	$34.7 \pm .597$
Difference		$9.08 \pm .924$

When the Flemish race is compared with the New Zealand Reds the difference in concentration is 12.98 ± 1.15 . This is more than eleven times the probable error. When the pure Flemish zygotes are compared with the Flemish-Polish hybrids, the difference is $9.08 \pm .924$, a difference of approximately ten times the probable error. From the data presented here, it is clearly evident that the genetic composition of the zygote controls in some way the concentration of glutathione in the tissues of newly born rabbits, analyses being made after the young have fasted 48 hours.

Let us again summarize briefly the independent studies which have a bearing on cell proliferation, some of which upon casual consideration may not appear to be connected with the problem of size inheritance. Hammett and his associates showed that the rate of cell multiplication is regulated by sulphydryl concentration. Painter (1927) found that the difference in size of the embryos of the large and small races of rabbit at twelve days of age is caused by a difference in cell number. Castle and Gregory (1929), and Gregory and Castle (1931) presented evidence showing that the rate of cell proliferation of eggs from races which differ in adult weight is definitely related to the adult weight of their respective races.

When the results of all these independent studies are considered together, they furnish strong evidence that at least one of the important biochemical substances involved in size inheritance and the regulation of body size is the concentration of glutathione in the body tissues.

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PRELIMINARY SYMBOLS FOR A TAIL-MUTATION
IN MICE

HEREDITARY transmission of the anoury and brachyury (complete or partial taillessness) observed in mice of French origin (Lang, 1912; Duboscq, 1922; Dobrovolskaia-Zavadskaia and Kobozeff, 1927) segregates as a simple unit, dominant over the corresponding normal condition and lethal in homozygous state: crosses between two abnormal animals give segregation in a 2:1 ratio, and crosses of an abnormal with a normal - 1:1 ratio.

Though it is possible that this mutation may turn out to be a chromosomal deficiency, the fact that it has its own phenotypic characteristics and behaves as a simple Mendelian character justifies its notation by a symbol *T* (tail-reduction), which has already been used for this mutation before.

The most striking effect of this gene is to reduce, in different degrees, the length of the tail and to bring about sometimes a complete disappearance of this organ. This gene may affect also the higher parts of the spine, which may result in paraplegia (Dobrovolskaia-Zavadskaia and Kobozeff, 1932).

A great variability characterizes this mutation, variability which concerns not only the length of the tail but also its form. A specific tail-length within the limits of a few neighboring vertebrae is very often transmitted from parents to offspring, as our radiographic examinations have shown. Some peculiar tail-forms which emerged during our breeding experiments in mice with this mutation proved their ability not only to be transmitted from parents to offspring, but also to be isolated by selection in separate lines. We have, for instance, a few lines of tailless and filiform-tailed mice which are breeding true (balanced lethal?).

These facts show that the gene *T* is acting in cooperation with some other factors (modifiers), which specify and diversify the effect produced by the main gene (*T*).

All this brought us to the idea that a list of *phenotypic symbols* for variations in tail length and form in the presence of gene *T*, established on the animals of French origin, might be suggested as useful in further investigations upon this mutation.

The shortening of the tail, which is the main feature of our mutation, is symbolized by the *T* itself. Since the degree of this shortening may be transmitted by heredity, the following

additional notations may be suggested to describe the appearance of the various types of tails: T v0 (v0 indicating that there are no caudal vertebrae at all) for complete taillessness (anoury); T v1, T v2 . . . T vn—for various lengths of short-tails (brachyury).

Further investigations must show whether the numerals 1, 2 . . . n apply to groups of vertebrae, or to single vertebrae. At any rate, since the normal mouse tail contains, at least in our stock, 30 vertebrae, T v30 would designate the normal condition. The lethal effect is connected with the gene *T*.

Some special forms, according to their ability to be transmitted hereditarily and selected in separate lines, need further notation. The most common of these forms is the "kinky tail." "Kink" ("Knickung" of Plate, 1910-13) was observed in mice as an independent mutation without any real shortening of the tail. "Flexed tail" in mice, described by Hunt and Permar (1928), presents also one or a few kinks of the tail, and it seems to breed as a Mendelian recessive character (Hunt, 1932).

Our radiographic study of short-tailed mice showed that there are two kinds of kinks in our mice: one depending on the insufficient growth from one side of a vertebra. We suggest signifying it with *k vrt*; and another—depending on the modification of a cartilaginous disk; this kind of kink may be represented by the symbol *k crt*. It seems that the mutation, studied anatomically by Blank (1916), belonged to this latter type.

A combination of T vn with *k vrt* and *k crt* may sufficiently describe all short kinky tails which we consider provisionally as one type of tail mutation, though it is quite possible that further selection will subdivide this group in a few different lines. A "filiform tail" (appendage devoid of skeleton) may be kinked as well as a bony tail.

A hereditary transmission of some other forms and their ability to be isolated by selection in separate lines were also established in our laboratory. We may suggest the following notations for these forms: for our anoure-filiform (*flf*) stock—a combined symbol T vo *flf*; for mice having a short tail with a filiform attenuated tip—T vn *flf*; for mice with an interruption of the axial skeleton due to a deficiency (*dfc*) of some caudal vertebrae T vn *dfc*; for mice manifesting a tendency to have their tails turned around as a helix (*hlx*)—T vn *hlx*, etc.

These symbols were composed according to current genetic practice (Report of the Committee on Genetic Form and Nomenclature, 1931; Bridges and Morgan, 1923); they are suggested as preliminary phenotypic ones, needing genetic verification on selected lines. But since other laboratories have begun work on these animals, we thought it timely to present these tentative symbols for common use in order to simplify the general conception of this mutation and to avoid perhaps in future unnecessary confusion of terminology.

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CONSTANT SPECIES HYBRIDS

A RECENT paper by Winge (1) describes twenty-four amphidiploid hybrids that have made their appearance in the literature of cytology and genetics in the past decade. In view of the fact that several erroneous impressions may be gained from this paper, which will be more widely circulated than one by the present author dealing with only eighteen amphidiploid hybrids, the following notes are submitted.

The first critical description and report of an amphidiploid hybrid was given in 1925 by Clausen and Goodspeed. These investigators had hybridized *Nicotiana tabacum* (24_{II}) and *N.*

glutinosa (12_{II}), and obtained in F_1 a fairly fertile hybrid which gave rise to a non-segregating F_2 . All members of this uniform generation showed the same chromosome number, which was the sum of the diploid numbers of the two parent species. This constant hybrid they named *N. digluta* (36_{II}). They were the first investigators who recognized the true nature of the derived hybrid, and at the time they called attention to the fact that *Primula kewensis* had probably had a similar origin.

The paper by Winge, now under discussion, by purporting to offer a chronological treatment of twenty-four such hybrids, would tend to create the impression that *P. kewensis* came first. This was doubtless done because *P. kewensis* was first noted as a constant hybrid about 1905. As a matter of fact at least two other constant species hybrids were known in the literature previous to 1900. In 1881 Focke in *Die Pflanzenmischlinge* mentions *Aesculus carnea*, derived from *A. hippocastanum* \times *A. pavia* as a frequently occurring constant species hybrid. Skovsted later proved this to be an amphidiploid. Janczewski (2) mentions another constant species hybrid obtained from the cross *Anemone sylvestris* \times *A. magellanica*. Therefore, neither chronologically nor in point of competent cytological analysis can *P. kewensis* be considered as the first known constant species hybrid.

Furthermore, one would get the impression from Winge that doubling of chromosome numbers in hybrids would automatically produce constant hybrids. Reports of the production of three such species hybrids in the genus *Crepis* (3) have been in the literature for some time, but Winge's paper omits them completely. Two of these proved completely sterile, and a third, which was somewhat fertile, was far from a constant amphidiploid hybrid. Winge's paper lists as constant several amphidiploids which the original investigators stated to exhibit irregular gametogenesis. For example, (1) *Triticum vulgare* \times *Secale cereale*; (2) *Digitalis ambigua* \times *D. purpurea*; (3) *Solanum nigrum* \times *S. luteum*; and (4) *Saxifraga granulata* \times *S. rosacea*. He does, however, mention the fact that the amphidiploid derived from *Nicotiana paniculata* \times *N. rustica* was inconstant. When this evidence is considered together with that of the three *Crepis* amphidiploids, it is apparent that chromosome doubling in species hybrids is not at all certain to result in constant species hybrids. In fact, it is evident that the phylogenetic relationship of the two parent species must be considered. If they

are closely related then allosyndesis will result in irregular maturation divisions, but if they are distantly related then what pairing does occur in the amphidiploid will be autosyndetic, and the maturation divisions will tend to be regular. The present writer has previously suggested that, with regard to maturation divisions, at least three kinds of amphidiploids may exist: (a) with no interspecific pairing, hence potentially constant hybrids; (b) with a minimum amount of interspecific pairing, hence with a possibility of producing stable derivatives; and (c) with a maximum amount of interspecific pairing, hence with little or no regularity in gametogenesis and little probability of producing constant derivatives.

It should be mentioned in conclusion that Levitsky and Benetzkaja (4), the investigators of the wheat-rye amphidiploid alluded to as showing irregular maturation divisions, have published a paper subsequent to the one originally cited by Winge and the present author. In this second paper they are at some pains in questioning the supposedly regular maturation divisions of many reported amphidiploid hybrids. Such caution appears to the present writer to be necessary in view of the accumulating evidence.

Also it should be mentioned that the present author (3) mis-cited a paper by Professor Huskins, of McGill University, "The Origin of *Spartina Townsendii*," which should have appeared as *Genetica*, 12: 531-538. 1930.

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VICINISM IN *AQUILEGIA VULGARIS*

SPECIES of *Aquilegia* can be crossed readily in controlled pollinations. How readily such inter-specific crosses might take place under natural conditions is a matter of general theoretical interest as well as of technical importance. In the summer of

1930 an opportunity presented itself for testing the amount of out-crossing in *Aquilegia vulgaris*, both within the species and outside the species. In the cultures of *Aquilegia* grown by the junior author at the John Innes Horticultural Institution, the following strains occupied successive rows in the bed:

- A. vulgaris*, dwarf red, double flowered
- A. vulgaris*, segregating for height, color, etc.
- A. vulgaris*, " " " " "
- A. Skinneri*
- A. pyrenaica*
- A. pyrenaica*
- A. caerulea*

The second family of *A. vulgaris* had resulted from a selfed heterozygote. It was segregating for dwarfness, for self-colored petals *versus* white-bordered petals, and for a recessive pink-intensifier which turned pale pinks into bright pinks, and blues into slaty-blues. It was also segregating for an albinotic or "tinged white" flower color whose factorial basis is not fully worked out. One of the few dwarf-slaty plants was chosen for the experiment. It stood in the third row between two tall blue-flowered plants. It was a homozygous recessive for dwarf and for the pink intensifier. It might or might not carry the "tinged white" and the white-bordered petal factors. The majority of outcrosses within the family could be detected since they would bring in either tall or pure blue.

Adjacent to the mother plant in the next row was a large plant of *A. Skinneri*, which previous experience had shown to cross readily with *A. vulgaris*. To make doubly certain a sister plant of the dwarf-slaty *A. vulgaris* was pollinated with *A. Skinneri* and the seedlings were raised to maturity. The plants of *A. pyrenaica* and of *A. caerulea* were farther away but they were in flower at the same time and bees were observed to fly freely back and forth between flowers of the different species. An effort was made to harvest all the seed from the mother plant. The capsules were carefully watched and harvested as soon as they started to open. When all the seed from the plant had been obtained it was thoroughly mixed and a portion was sown in 1931. Care was taken to prick off the seedlings as soon as they appeared in the seed pans. The pans were kept watered for three months after the last seedlings appeared in the hope of catching any weak hybrids which might be late to germinate.

The plants flowered in the spring of 1932. They were all pure *A. vulgaris*. If inter-specific crosses had occurred they had been

eliminated in some way. The seedlings were of the following color and growth types:

	Dwarf	Tall
<i>Self colored</i>		
slaty-blue	53	0
blue	0	10
<i>White edged</i>		
slaty-blue	17	0
blue	0	3
<i>White tinged</i>	30	6
Total	100	19

It will be remembered that the mother plant selected for the experiment was homozygous for dwarfness and for slaty color. The dwarfs were all slaty and segregated for white-edged petals in numbers very close to expectations on a three to one ratio. Since there were no other slaty-dwarf plants near-by it is probable that the 100 dwarf plants resulted from self-pollinations. The other 19 must have resulted from cross-pollinations in the same family. In flower color and pattern they correspond to what would have been expected—upon pollinating the dwarf-slaty mother plant with the two adjacent tall blues in the same row.

To summarize the evidence: there was a minimum of 16 per cent. (19 out of 119) of out-crossing within *A. vulgaris*, probably with adjacent plants. Out-crossing with the dwarf red-double *A. vulgaris* two rows away did not occur. There was no effective out-crossing with any of the other three species though they are known to be inter-fertile in controlled pollinations. Cross-pollinations with these three species probably occurred but were ineffective under the conditions of the experiment, due to some such factor as differential pollen-tube growth or embryonic competition.

SUMMARY

In an experiment planned to test the amount of out-crossing in *Aquilegia vulgaris* it was found that effective out-crossing with other species did not occur. Out-crossing within the species was found to have taken place in at least 19 out of 119 cases (16 per cent.).

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THE EFFECTS OF HEREDITARY AND ENVIRONMENTAL DIFFERENCES UPON HUMAN PERSONALITY AS REVEALED BY STUDIES OF TWINS¹

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IN this discussion I do not propose to broach the question: Which is more important, heredity or environment? Such a question is meaningless in the raw form. The question I do propose to ask is quite different—With regard to certain physical, mental and temperamental characters in man, what are the relative potencies of hereditary differences and of environmental differences in determining the end result?

If it were possible to control human breeding so as to produce pure lines and to subject part of a pure line to one type of environment and part to another, it would be relatively easy to determine to what extent differences are genetic and to what extent environmental. In the present state of society no such ideal type of experiment is likely to be permitted for a long time. Therefore, we must resort to other methods of investigating the factors of heredity and environment. Fortunately, the study of resemblances and differences in twins offers us a very good method of isolating the inherited aptitudes from the influence of environmental differences in certain

¹ Paper read at the symposium of the American Society of Naturalists on "Heredity and Environment in Man," Atlantic City, December 30, 1932

human beings. In other words, twins afford an almost complete substitute for a controlled scientific experiment.

Let me explain. Identical twins reared together constitute a control group in which, for each pair, heredity is the same and environment as nearly as possible the same. Fraternal twins reared together constitute one experimental set-up. Fraternal twins have had a common prenatal and a common post-natal environment. They differ from the control only in one factor, namely, heredity, and hence any greater differences found in the experiment than in the control may be attributed to the varying factor, differences in heredity.

Fraternal twins, however, do not give the heredity factor a fair chance to show its potentialities, for fraternal twins are closely related genetically, being brothers or sisters, and are therefore much more similar genetically than would be pairs of persons of the same race chosen at random. I think it would be fair to both heredity and environment to consider fraternal twins as on the average 50 per cent. genetically similar and 50 per cent. genetically different. Even this estimate probably favors the environment factor and disfavors the heredity factor. If then only a 50 per cent. hereditary difference produces certain effects it would be fair to say that a random assortment of unrelated individuals would show twice the effects observed in fraternal twins reared together.

The converse experiment to that just described is one in which the heredity of two individuals is the same, but the environment differs in various ways. Such a set-up is afforded by cases of identical twins separated in infancy and reared apart under different environmental conditions. Greater differences found in separated twins than in those reared together may safely be attributed to differences in the environment.

Realizing the possibilities of substituting twins for controlled experiments, Drs. Freeman, Holzinger and I began about five years ago a program of twin studies.

So far we have studied 50 pairs of identical twins reared together, constituting the control; 50 pairs of same-sexed fraternal twins reared together, constituting the material for testing the potency of hereditary differences; and 10 pairs of identical twins reared apart, constituting the material for testing the potency of environmental differences.

A necessary preliminary to such study of twins is a reliable technique for diagnosing twins as identicals or fraternal. A complete account of our elaborate technique of diagnosis would be impossible in the time permitted. Suffice it to say that we believe we have made few, if any, mistakes. Our statistician, Dr. Holzinger, claims that as many as five mistakes in diagnosing the original 100 pairs of twins would not alter the findings to a significant degree. Let us assume, then, that it is possible to diagnose identical and fraternal twins with sufficient accuracy for our purposes.

All these twins have been extensively studied and measured for physical, mental and temperament-emotional differences, and we have in hand a very large mass of data. Only a bare outline of these data can be given to-day.

A great many physical characters, such as hair color, hair form, eye color, skin texture, distribution of body hair, stature, general body build, shape of hands and feet, ear shape, facial features, shape, size and arrangements of the teeth, etc., are no more different in identical twins reared apart than in those reared together. Such characters, then, are not modified by the existing differences in the environment and may be considered as purely hereditary.

There are, however, some characters in which identical twins reared apart are much more different than identical twins reared together. Such characters may be thought of as partly controlled by differences in the environment. It is only such characters as these that afford an opportunity for testing the relative potencies

of hereditary and environmental differences. Some of these characters are body weight, general physical health and strength, condition or state of preservation of teeth, mental rating in terms of Intelligence Quotients (IQ), temperament-emotional status, as determined by differences in overt behavior and by standard psychological tests. Greater average differences found in identical twins reared apart than in those reared together may be considered as a measure of the effects of differences in environment. These differences may then be compared with differences due to genetic differences as seen in fraternal twins reared together.

In this way we should be able to determine which of the two kinds of differences, hereditary or environmental, produce the larger effects as compared with the control, identical twins reared together. Let us see how this comparison works out with certain sample characters, taking as an example of physical characters body weight and as an example of mental characters the Intelligence Quotient (IQ).

The mean differences in *body weight* for the three groups of twins are as follows:

(1) Identical twins reared together	= 4 lbs.
(2) Identical twins reared apart	= 10 lbs.
(3) Fraternal twins reared together	= 10 lbs.

The two experimental groups, 2 and 3, both show $2\frac{1}{2}$ times as great a mean difference as that of the control. This might be taken to mean that differences in environment have a potency equal to differences in heredity, were it not for the fact that fraternal twins are at most only 50 per cent. different in heredity. Since a 50 per cent. difference gives the same effect as the whole of the environmental differences found in 10 pairs of identical twins reared apart, we may conclude that hereditary differences are about twice as influential as are environmental differences in determining the physical character, body weight.

Much the same situation is found when we study variations in IQ. The mean difference in IQ (Stanford-Binet Test) for identical twins reared together is 5.3 points; that for identical twins reared apart is nearly 9 points; that between fraternal twins reared together a little over 10 points. Roughly speaking, then, differences due to heredity and differences due to environment are both nearly double those in the control, though differences due to heredity are a little greater in their effects than those due to environment. Here again our determination of the effects of differences due to heredity are based on fraternal twins, which are no more than half different in genetic constitution. Once more we shall have to conclude that if a group showing hereditary differences of no more than 50 per cent. gives a certain effect, a group made up of randomly chosen pairs reared together would be expected to show twice as great a mean difference in IQ. From this we may conclude that hereditary differences are about twice as effective in determining differences in IQ as are the differences in environment in the twins reared apart.

Temperament-emotional differences are less easily measured than are body weight and IQ, but there is abundant evidence that heredity and environment have about the same relative effects upon temperamental traits as was the case for body weight and IQ. One indication that this is probably true is seen when we arrange differences in these three characters into three groups, such as great, moderate and slight, and see how many of the ten cases of identical twins reared apart fall into these three classes. For body weight 4 pairs show great, 4 pairs moderate and 2 pairs slight differences. For IQ 4 pairs show great, 4 pairs moderate, 2 pairs slight differences. For temperament-emotional traits 4 pairs show great, 3 pairs moderate and 3 pairs slight differences. The distribution is nearly the same for all three traits, an indication that they are about equally effected by differences in heredity and those in environment.

So far as our materials go, we may say, then, that the actual differences in heredity have about twice as great an effect as do the actual differences in the environment in producing the differences observed in such characters as body weight, IQ and temperament. This does not mean that heredity is twice as important as environment.

Now the rest of my time shall be devoted to a brief description of the ten cases of identical twins reared apart. This experimental group is at once the most critical and the most difficult to secure. Twins reared together are numerous, but identical twins reared apart constitute one of nature's rarest experiments. Through constant effort and at very considerable expenditure of money, tact, detective ability, and not without much sorrow and disappointment, we have in five years succeeded in getting the data on ten pairs. In every case the twins were separated in real infancy and kept apart for from 13 to 55 years. The environments of the various separated pairs differed markedly in nearly all the cases. It is difficult to imagine environmental differences much greater than those found, unless one were to separate twins in such a way that one was reared in one country and the other in a very different country. It is, however, necessary to have them reared in the same cultural complex; otherwise they could not take the same psychological tests. On the whole we feel that the differences of environment found in these cases represent the normal range of environmental differences likely to be found among people of the same country and speaking the same language. It is doubtful whether a larger number of cases would very greatly modify the size of the mean differences already found, for the mean of the first five is almost exactly the same as that of the second five. If it is true that our ten cases are truly representative, we do not badly need any more cases. I am sure, however, that we shall not stop with ten cases and I hope others will help in contributing cases of their own.

Every case studied is perhaps more interesting and significant as a case study than as an item in a statistical summary. I am therefore going to describe briefly for you the separate cases, showing photographs of each pair. It will be noted that while a pair may differ greatly in one respect there may be only moderate or slight differences in other respects. Also it will be seen that wherever educational differences are very great there is a correspondingly great difference in IQ, wherever the differences in social environment are very great there are great differences in temperament and sometimes in IQ, and that wherever the differences in physical environments are great, there are great differences in physical condition. Slight differences in any of the three features of the environment seem to have no measurable effect.

Case I. Twins "A" and "O" were born in London, England, "A" living there until shortly before we examined them, while "O" was brought to Canada at eighteen months and reared in a medium-sized Ontario town. Quantitatively there was not much difference in their formal education, but there was a great difference qualitatively. "A" studied chiefly domestic subjects, while "O" had a well-rounded high-school course, followed by a business course. The war period in London seriously interfered with "A's" education, while "O" was little disturbed. The physical environment of "A," especially as regards food, rest and sleep during the war period, was in striking contrast with that of "O," living in the quiet, prosperous Ontario town.

At the time of the examination there was a great difference in their physical condition, "O" being much superior, their weights, "A" 92½ pounds and "O" 102 pounds, being diagnostic of their relative condition. In all the intelligence tests given "O" was definitely superior, the difference in IQ in the Stanford-Binet test being 12 points, nearly 2½ times the mean in the control group. Temperamentally, they were remarkably similar, the differences being only slight.

Case II. Twins "E" and "G" were born in New York City, were separated at eighteen months and reared unknown to each other in two Michigan cities. After twenty years of separation they found each other in a purely fortuitous manner, and had been living together for about two years when we studied them. There was no great difference in their physical or social environments, but a very great difference in formal education. "E" went through grade school, high school and two years of normal school, while "G" stopped school after the fifth grade in order to help with housework. At the present there is only a very slight difference physically: in fact they

are as similar as any of the pairs of identical twins reared together. There were 12 points difference in Stanford-Binet IQ in favor of "E," the more highly educated twin, and even greater differences in other intelligence tests. Temperamentally they are most strikingly similar. The fact that one is now a doctor's assistant and the other a dentist's assistant, and both eminently satisfied with their jobs, indicates that their personalities are nearly identical.

Case III. Twins "C" and "O" were born in an Illinois village and were early put in an orphans' home, from which they were soon adopted by two families unknown to each other. The twins were entirely separate for twenty years and met for the first time when they were twenty-one years old. They had met only once before when they came to Chicago to be studied. The chief environmental difference was one of city life *versus* rural life. "C" had always lived in rather large cities in Michigan, while "O" had been a village boy in various parts of Illinois. Though living in a rural community he had not engaged actively in farming, but had done mostly clerical work. They both had a high-school education and very little more.

When examined, there was a moderate difference physically, "C" being in better physical condition, better groomed, and weighing 124 pounds as compared with 113½ pounds for "O." There was no significant difference in mental rating between them, one ranking a little higher in one test, the other in another test. The scores on the Stanford-Binet test were: "C" 99 points and "O" 101 points; while on the Otis test "C" had a score of 110 and "O" 100. On the whole "C" made somewhat higher scores. Temperamentally there was a great difference. It appears that they were no more alike in their emotional reactions than would be two persons chosen at random.

Case IV. Twins Mary and Mabel were born in Ohio about thirty years ago, were separated at five months and reared by relatives. Mabel has lived all her life on a prosperous farm, living the life of an active farm woman. Mary has lived largely a sedentary life in a small town, clerking in a store during the day and teaching music at night. Mabel stopped school after the grade school, while Mary had a full high-school course in an excellent city school near her home. Mabel has always been very healthy, while Mary has been troubled a great deal with a series of severe colds every winter.

At the present time there is a vast difference between the twins in all three respects. Physically, Mabel is robust, muscular and in perfect health, while Mary is much under weight, soft muscled and in poor general condition. Mabel weighs 138½ pounds, Mary only 110½ pounds, a difference of nearly 28 pounds, about 25 per cent. of the weight of Mary. Intellectually the difference is equally striking, but in favor of Mary, whose Stanford-Binet IQ was 106, as compared with 88 for Mabel. The difference of 18 points is nearly 3½ times as great as the mean of the control, and the largest found in any identical twins studied. The differences were even greater in some of the other tests. Temperamentally these twins may be adjudged at least moderately different, being almost as different in most reactions as was true for the twins of Case III. Yet in a few respects there is an underlying resemblance that stands out sharply.

Case V. Twins "B" and "D" were born in Iowa of extremely young parents who were unable to keep them. They were put in a children's home at an early age and were separated at 14 months, never to meet again till they were sixteen. After a dramatic discovery of each other they lived together for about a year, when "D" married and moved away. There was no marked difference in the physical environment of the twins up to this time. Also their formal education was nearly equal, "B" finishing eleventh grade and "D" tenth grade. The chief environmental difference came after "D's" marriage. She married a poor man, who failed to make a living in Iowa and moved to the far West where he staked a claim and lived the hard, meager life of a pioneer. During this period "D" had four children in rapid succession and two more after moving back East. During the pioneer days food was poor and ill suited for a child-bearing woman. "B" some years later married a well-to-do merchant and has lived a relatively easy life, with domestic help and abundant social opportunities. She has had four children rather well spaced in time.

When examined at the age of 38, these twins showed great physical differences. "B" seemed unusually well preserved, looking hardly over thirty. Her teeth were absolutely perfect and her weight was 110½ pounds. "D," however, looked fully her age. Her teeth were in extremely bad condition, several having been extracted, others badly decayed, and the incisors discolored and badly worn. Her weight was 101 pounds.

Intellectually there is only a slight difference between them, "B's" Stanford-Binet IQ being 93 and "D's" 89. They class as moderately different temperamentally, though their resemblances are more impressive than their differences.

Case VI. Twins Ada and Ida were born fifty-eight years ago in a small town in Ontario and were adopted by two families of relatives when they were three years old. The early life of both twins was equally wretched. Apparently they were unwelcome in their foster homes and were badly abused. They had practically no formal education, attending country school irregularly for only about three years. In school they did not even learn to read. The moral influences in their homes were so bad that they themselves sometimes wonder how they ever grew up to be good women. They were entirely separated for thirteen years. Ada married at seventeen and Ida at thirty-three. Ada has reared five children, Ida four. Ada left her husband after four years, Ida still has hers. Ada has lived partly in the country, but of late mostly in large cities; Ida has always lived in the country.

When examined at the age of 58 these twins were very different physically, owing largely to the fact that Ida has an advanced goiter and was weak and inactive. Ada was strong and active and had no goiter. Ada weighed 208 pounds, Ida 227, a difference of 19 pounds. Intellectually both women made a surprisingly good showing considering the lack of schooling, Ada being moderately superior on all tests, her IQ being 101 to Ida's 93. Temperamentally they were remarkably similar. Both women are now militantly moral, probably as a reaction against childhood influences.

Both are highly respectable and highly respected women. They illustrate the ability of good heredity to surmount bad environment.

Case VII. Twins Richard and Raymond were born in Indiana and separated at one month of age, Raymond being adopted by a prominent physician in a rather large city and Richard by foster parents in less favorable circumstances. Raymond stayed at home and was more sheltered, while Richard was taken about far more, was more thrown on his own resources, and also saw more of the seamy side of life. They had exactly the same amount of schooling and were at $13\frac{1}{2}$ years of age in the same grade, the eighth grade, both among the brightest students in the class.

When examined at the age of $13\frac{1}{2}$ years, there was no difference of any consequence physically. The differences in IQ for all tests were slight, the scores for the Stanford-Binet test being 106 for Richard and 105 for Raymond. Temperamentally, they were moderately different. Richard seemed more positive in all his characteristics and gave a much more normal reaction to such tests as the Kent-Rosanoff Association test. Hence the only significant difference between these twins is a moderate difference in temperament.

Case VIII. Twins "M" and "R" were born in Indiana and were separated at three months, "M" being adopted by a maternal uncle, and "R" by a brother of "M's" foster mother. "M" was reared in a town of 5,000 population, where she knew nearly everybody in town and had many friends and playmates. Her foster father was a leading citizen, well educated and had a cultured home, involving good books, good music, etc. "R's" home was relatively narrow and unstimulating. Neither of her parents had much education and, although living in a large city, she had but few friends and made but few social contacts. In fact, she was kept closely at home. The environmental contrast is that of a stimulating and cultured home environment *versus* a depressing and uncultured home environment. There was very little difference in the schooling of the two girls, "M" attending school at home and being now in grade 10A, while "R" has attended a good city school and is in 10B. The physical environment has been about the same for the twins.

When examined at about 15 years of age the girls were remarkably similar physically. There was a great difference in intelligence, "M" being distinctly superior in all tests, "M's" score on the Stanford-Binet test being 92 and "R's" 77, a difference of 15 points, greater than that found in Cases I and II. This great difference must be the result of differences in informal education. Temperamentally the differences are great both in overt behavior and in reactions to tests. "R" is extremely timid and retiring. She lispes markedly and seems unhappy. "M" seems quite normal in behavior. The tests all show about the same contrasts.

Case IX. Twins Harold and Holden were born in Wisconsin and separated in early infancy, but have lived only three miles apart. Harold has lived in a village and has not done much manual labor, while Holden has lived on a dairy farm and has worked ever since he was old enough to be useful. They attended different grade schools but were graduated from the same high school, Holden one year ahead of Harold. Harold has had much

more opportunity to engage in group activities, while Holden has been denied this. The differences in environment have not been at all marked, less so than any other of our cases.

When examined at 17 years of age these boys differed slightly in all respects but were almost as similar as the average pair of identical twins reared together. The Stanford-Binet test gave Harold a score of 102 and Holden a score of 96. Harold was somewhat superior in all other mental tests. Holden was somewhat superior physically in most respects, but both seemed to be in perfect physical condition. Temperamentally, the differences were rather marked in some respects, Harold appearing rather more normal. On the whole it is necessary to diagnose all three types of differences as "slight," though the mental differences are almost "moderate."

Case X. Twins "B" and "J" (described by H. J. Muller). This case was the first one studied and should have been described first, but my cases have already been numbered from I to IX. These twins were born in a South Dakota mining camp and were separated at two weeks. "B" was brought up by foster parents engaged in mining and hauling. She had four years of formal schooling and nine months in a business school. Since she was fifteen she has had an active business career, being in France during and after the war. Her life has been more varied and rougher than that of "J." "J" was reared by well-to-do parents who owned a ranch and a tourist hotel. She went through grade school, high school and attended some summer sessions at the university. She is a school teacher by profession, though she married at 21 years of age and has one son.

When examined at 27 years of age, these twins were nearly identical physically. They were also nearly identical mentally, "B" having an IQ of 156 and "J" of 153, both very high, "B" the less educated twin having the higher score. Temperamentally they appear to be no more alike than two women chosen at random.

It is fully realized that these brief summary descriptions of the ten cases are entirely inadequate, but they serve to bring out a few of the salient facts. Case studies of Muller's case and of our first six cases have already been published in the *Journal of Heredity* and Cases VII, VIII and IX are now in press in that journal. At a later time Drs. Freeman, Holzinger and I expect to publish, probably in book form, as complete an analysis of these ten cases as we are able to make. Any conclusions stated in this and other papers already published must be considered as tentative and subject to alteration should further analysis warrant it.

In the descriptions of the ten cases just given more emphasis has been placed upon differences than upon re-

semblances. This has seemed necessary in order that such differences as do exist may be brought out at all. We have, for example, considered differences in IQ of 12 points or over (Cases I, II, IV and VIII) as great differences, though I am well aware that psychologists claim that differences of this magnitude may occur when a given person takes the same test twice. The largest difference found was one of 18 points, which psychologists do not consider as more than a minor one. Differences of these dimensions assume greater significance, however, when it is stated that the majority of identical twins reared together show differences of from 0 to 3 points in IQ, and that the mean difference of 5.3 points is produced by half a dozen exceptional pairs that show differences of 10 points or over. There is no reason, however, for insisting that the observed differences in IQ or in other respects are really great. Our only concern has been to give a fair emphasis to the actual differences observed. Any over-emphasis upon the effectiveness of environmental differences is certainly corrected when the mean differences with regard to the various traits is determined.

As previously shown, these mean differences for the separated identical twins turn out to be approximately the same in extent for body weight, IQ, and perhaps also for temperament, as the mean differences found in fraternal twins reared together. What interpretation may be placed on these figures? On the assumption *a*, that one fraternal twin in a pair differs from the other on the average about as much as would two persons chosen at random, and *b*, that the environmental differences encountered by two identical twins reared apart are on the average about as great as those encountered by two persons chosen at random, hereditary and environmental differences might be said to have about equal potencies. These two assumptions, however, demand scrutiny. The first assumption is obviously unjustified, for it can be shown in a great variety of ways that the hereditary dif-

ferences between fraternal twins are no greater than between sibs and that sibs differ on the average about half as much as do persons of the same race chosen at random. If this be granted, it would follow that if hereditary differences were given full play the effect of these differences would be about twice as great as those found in fraternal twins reared together. Since the mean differences found in fraternal twins reared together are at least equal to those of identical twins reared apart it would be fair to conclude that randomly paired individuals reared together would show a mean difference twice as great as that found in identical twins reared apart. The second assumption may also be unjustified, for we have no assurance that the actual differences of environment between these separated twins average as great as those likely to be found between pairs of individuals chosen at random. It seems to me, however, that the actual differences in environment in these ten cases represent about the full range of such differences likely to be encountered by persons of the same race living in the same country. Our conclusion stands or falls on the basis of the justifiability of this assumption, and has to do with such modifiable characters as body weight, general health and physical condition, IQ, and temperament-emotional traits. There can be no doubt, of course, that more extreme environmental differences than those encountered by our twins reared apart would result in proportionately greater differences. All that our studies of twins tend to show is that, in a given country, the actually existing hereditary differences are likely to be about twice as effective in determining the exact status of certain modifiable personal characteristics as are the environmental differences likely to be encountered.

NEEDED EVIDENCE¹

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THE problem of determination of mental ability through the influences of heredity and environment has been recognized as a fertile research field since Galton in 1869 published his much quoted study of "Hereditary Genius." Though nature and nurture each had strong advocates dating from the time of Plato and before, it was not until Galton's pioneer work that an effort was made to view the problem scientifically and to advance beyond speculation, uncontrolled observation and anecdote. Now, thanks to the mass of careful work which has been accumulating since Galton's time, and particularly during the last ten years, it is possible to accept certain conclusions with reasonable assurance that they represent what is true rather than what could conceivably be true. However, there are still certain controversial issues—hence the symposium of which this paper is a part.

The present paper is limited chiefly to a discussion of evidence regarding intelligence, the mental trait concerning which the most data are available, and about which there should be correspondingly the least controversy. Parenthetically, it may be said that there is cogent reason, both *a priori* and by the results of experiment, to believe that personality traits are more influenced by nurture than are the more intellectual variety of mental traits. We must distinguish between what a person *can* do—the difficulty of mental performance which he is able to attain (intelligence) and what he *will* do—which of the many courses of behavior within his physical and mental capacity he will characteristically take (personality).

¹ Paper read at the symposium of the American Society of Naturalists on "Heredity and Environment in Man," Atlantic City, December 30, 1932.

HISTORICAL ADVANCE OF PROBLEM AND METHODS OF APPROACH

The history of the subject has more than ordinary interest because all the historic approaches are still currently used, sometimes, to be sure, to the discredit of the field.

As in other developments in science, a typical sequence can be observed, beginning with the gross drift out of which an originator like Galton could discover a pattern, and ending with crucial, quantitative work. We may use such a device as is much in favor by geneticists for illustrating the sequence of results in terms of the techniques employed to obtain them. Listed vertically are some

HISTORICAL DEVELOPMENT OF NATURE-NURTURE PROBLEM Method

Status of knowledge	Study of ability of family lines	Biometric study of family resemblance	Biometric study of twin resemblance	Statistical study of ability in social and racial groups	Experimental technique	Special studies of data on family resemblance
A. Definition of the problem	X	X	X
B. Unquantitative determinations:						
Influence of heredity	X	X	Orphans
Influence of environment	X	Animals
C. Quantitative determinations:						
Estimates of ability	X	X
Influence of heredity	Co-twin control; twins reared apart	Foster children	X
Influence of environment	Co-twin control; twins reared apart	Foster children	X
D. Mechanism of heredity						
Mode of transmission	Animals	X
Linkage	X

rubrics indicating the progressive status of our knowledge, while horizontally are listed the modes of attack. The crosses and notations in the checkerboard show the relationship between the types of data collected and the types of knowledge they contribute. We may take up for discussion one by one the various types of available data (horizontal list) and examine the residues they leave.

STUDY OF HIGH AND LOW ABILITY IN FAMILY LINES

The early work of Galton set the pattern for this type of study; and until about 1900 it was virtually the only kind carried out. Counts were made of the eminently able individuals of certain family stocks, and of the defective individuals of others. Casual observations of the facts of family resemblance such as any one might make were thus replaced by statistical formulations of the tendency for one or the other extreme of ability to cluster in family lines. The eminent men studied by Galton, the gifted Jonathan Edwards clan (Winship, 1900), and the notorious aments and paupers of the Jukes (Winship, 1900) and Kalikaks (Goddard, 1912) of America furnish such dramatic material that their histories have been and still are summarized in practically every text-book touching upon mental heredity in man. Such studies are designated on our checkerboard as defining the existence of the nature-nurture problem. They permit in a limited way an estimate of the ability of the relatives of an individual of great or meager ability. Beyond this, however, they do not go, for there is nothing within such data enabling us to untangle the effects of nature and nurture. Moreover, their value for prediction is extremely limited. It is of little general usefulness to be able to say that a man of genius is 134 times as likely as a random man in the street to have an eminent relative.

BIOMETRIC STUDY OF FAMILY RESEMBLANCE

Pearson, with his series of correlation studies initiated in about 1900, set the pace for a large group of subsequent workers. Pearson's studies were based upon trait

ratings, whereas later studies have been facilitated by superior measures from mental tests.¹ The correlation between mental ability of relatives of the first degree (parents and offspring, brothers and sisters) has been established as about .50, an amount which checks well with the correlations found for hereditary physical traits, and which checks also with the theoretical expectation on a Mendelian hypothesis of cumulative, non-dominant genes. These studies are of value in making possible predictions of the ability of individuals whose parents or other relatives are of known mental level, but do not enable us any more than do the "incidence" studies to separate nature and nurture and attribute the observed facts to one or the other. An environmental hypothesis rather than one of Mendelian inheritance might conceivably account for the correlations.

BIOMETRIC STUDY OF TWIN RESEMBLANCE

A good many investigations of twins have accumulated since Galton himself saw the need for something more informing than his own "incidence" studies. He collected data (1883) showing that twins of the identical or uniovular type tend to remain very similar throughout life, while twins of the fraternal or biovular type tend to retain the dissimilarities with which they start in childhood. The later studies (particularly by Merriman, 1924; Lauterbach, 1925; Wingfield, 1928; Holzinger, 1929; Hirsch, 1930) express the resemblance of identical and fraternal twins in terms of correlation coefficients. They show that pairs of identicals resemble one another almost as much as an individual resembles himself when tested twice with a short time intervening (about .90), while pairs of fraternal twins resemble one another little if any more than do ordinary siblings (about .50).

This type of study has been designated on our checkerboard as demonstrating the influence of heredity, since

¹ Some of the most extensive family resemblance studies have been carried on by Rensch, 1921; Hart, 1924; Hildreth, 1925; Jones, 1928; Burks, 1928; Thorndike, 1928; Willoughby, 1930.

the children with identical heredity are much more alike on the average than are those whose hereditary potentialities are determined by different gametes. It is hardly possible, however, to draw any quantitative conclusions from such data, even though the correlations give the illusion of precision. We do not know to what degree environment may have contributed to the resemblances of both types of twins. The several formulas which have been proposed for appraising on the basis of correlations the relative contributions of nature and nurture are ambiguous, since they assume the environments of the two types of twins to be equally equal, an assumption subject to unknown error. (See preliminary study of differential environmental differences by Wilson, 1932.) Moreover, even if these formulas could accurately separate nature and nurture contributions to twin pair differences, this would be without general interest, since inferences can not be drawn therefrom regarding the causes of variability among individuals at large.

From studies of co-twin control (Gesell and Thompson, 1929; Strayer, 1930), and of twins reared apart (Muller, 1925; Newman, 1929-1932), really quantitative data may be ultimately expected. The number of cases of either kind is not yet sufficient to warrant definite conclusions, though it is significant that the mental test scores reported for ten sets of identical twins reared apart show a somewhat greater average discrepancy than is typically found for twins reared together. One difficulty in studies of twins reared apart is that members of a pair are often placed selectively, occasionally even with close relatives. The obtained results are therefore only minimum estimates of the mental disparities which truly random environmental differences might be expected to yield.

STATISTICAL STUDY OF ABILITY IN SOCIAL AND RACIAL GROUPS

Studies of mental differences among people of different social, educational or racial groups have sometimes been

cited as evidence for the influence of heredity. The position might better be taken that the existence of such differences are simply additional indication of the nature-nurture problem itself, to be examined with the same caution and subject to the same pitfalls as are other indications of the problem. The writer knows of no really crucial studies upon race differences in intelligence. There are several studies, however, which suggest a measurable effect upon mental development of extreme educational deprivation, and several which suggest that ordinary differences in education or cultural opportunities have little effect upon differences in mentality. Children who lived all their lives upon canal boats in England (Gordon, 1923), showed a pronounced inverse correlation between age and mental standing. On the other hand, little relationship has been found between total school attendance and IQ among school children of a given age in urban communities (Heilman, 1928; Denworth, 1928).

Although a number of investigations show rural children to average distinctly lower in IQ than do city children, two studies made in England indicate that this difference is probably largely native, and due to selective migration of the higher levels of ability to the cities. The English investigators found as others had before them a lower average mental level among children in ordinary rural settlements. However, children in very remote rural areas, where little "drainage" to the cities had taken place, averaged as high in ability as city children. (Bickersteth, 1919; Duff and Thomson, 1923.)

Additional light upon the origin of mental differences associated with social status may be found in several of the studies summarized in the next section.

EXPERIMENTAL TECHNIQUE

It is probably a safe generalization that a field of investigation becomes scientific in proportion as experimentation becomes possible and is utilized. In the field with

which we are concerned the studies which resolve rather than stimulate controversy are nearly all of an experimental kind, though often the experiment is set and conducted by certain agencies of our social structure whose aims are remote from research. As psychologists, biologists or sociologists, we are sometimes able to step in and measure the results of such experiments. This has been done particularly with children reared in orphanages and with foster children. Studies of twins reared apart might also be mentioned here as a special case of the foster child approach, though it seemed more appropriate to discuss the significance of such studies in the general section on twins, p. 209, *ff.*) Gesell's co-twin control studies are among the few involving actual experimentation by the investigator, but were classified also in the general section on twins.

In the studies of orphanage children, living of course in a relatively uniform environment, it is extremely interesting that differences persist in the average IQ's of children whose parents were of varying occupational or educational levels. (Jones and Carr-Saunders, 1927; Lawrence, 1932). Although children of differing origin do show somewhat smaller differences than usually appear between corresponding groups of children reared in their own homes, it is not known whether this fact must be attributed to a leveling influence of orphanage life, or to selection as to the type of dependent child who receives institutional placement.

Several studies which show the correlation between the IQ's of siblings reared in orphanages to be almost, if not quite, as high as between siblings reared in their own homes give rather cogent evidence for the influence of heredity, although the possibility is not ruled out that home and family influence in early childhood years may have made at least some contribution to sibling resemblance. (Gordon, 1918-20; Hildreth, 1925; Davis, 1928.)

Investigations employing animals have a great advantage over those employing human subjects, in that all

may be reared under the same carefully controlled conditions. Except for such small effects as may be assigned to inter-uterine environment or to nutrition in the early weeks of life, the family resemblances which several investigators have found in the maze-running ability of albino rats must be attributed to nature (Tolman, 1924; Burlingame and Stone, 1928; Tryon, 1929). In the Tryon studies a very significant hereditary effect has been demonstrated through eight generations of selective breeding for "brightness" and "dullness" in maze-running. Two strains have been developed with practically no overlapping of ability. The strains offer extremely valuable material for testing various hypotheses as to the mode of transmission of the particular mental trait measured, and further work is planned by Tryon toward this end.

It is through studies of foster children adopted in infancy that we should be best able to obtain a quantitative determination of the effects of nurture, and by inference, the complementary effect of nature. There are three large-scale studies of foster children (Theis, 1925; Burks, 1928; Freeman, Holzinger and Mitchell, 1928), though only the second and third employ mental tests. These two, conducted at Stanford and Chicago, respectively, agree in certain important respects, and disagree in others. Let us consider the main findings in the light of methods used.

Groups studied: The Chicago group contained various subgroups aggregating about 400 children in all. The group was heterogeneous with respect to race and age of placement, and ranged (with a few exceptions) from three to eighteen years at time of test. In the Stanford study subjects consisted of about 200 foster children and 100 control children reared with their own parents. The subjects were all of white "American" or North-European stock, so chosen in order to guard as far as possible against selective placement. The children ranged from five to fourteen years at time of test. All were placed when under a year of age.

Comparative Methods and Results: IQ's of children in both studies were measured on the Stanford-Binet. Mental ages of adults were measured on the Stanford-Binet in the Stanford study, and on the Otis in the Chicago study. A composite environmental home rating was obtained by somewhat different methods in the two studies, though each was concerned both with the material level and the cultural standards of the home.

Comparative correlations:	Stanford	Chicago
IQ of foster child vs. MA of foster father07	.37
IQ of foster child vs. MA of foster mother19	.28
IQ of foster child vs. environmental rating39	.48
IQ of child vs. MA of own father45
IQ of child vs. MA of own mother46

That the higher correlations found in the Chicago study may be due at least partly to selective placement rather than to environmental influence is suggested by the results from the Chicago "pre-test" group of children who were old enough to test before entering foster homes. Their IQ's before placement correlated .34 with the environmental rating. After an average foster home residence of four years, IQ's and environment correlated .54. This increase, while showing a real if relatively small effect of environment, brings the correlation to only a little above the value found for the children who were too young to test at time of placement. It therefore seems a fair inference that some degree of selective placement influenced the results for the entire Chicago group. Miss Leahy (1932) in her study of Minnesota adoptions found appreciable evidence of selective placement.

Other significant findings in the Chicago study were:

A correlation of .34 between the IQ's of 125 pairs of siblings reared apart. This correlation becomes .44 if only white children between the ages of five and fourteen are used, and is then approximately equal to that found for siblings reared under normal circumstances.

A correlation of .31 between the IQ's of 112 pairs of foster siblings, *i.e.*, unrelated children reared in the same home. It is probable that this correlation is partly due to selective placement.

An average increase of about 7 points in the IQ's of the pre-test group after four years of residence in good foster homes.

Conclusions: The authors of the Chicago study concluded that environment was an important factor in their results. The one really quantitative appraisal of the effect of environment was made in the pre-test group, which showed an average increase in IQ of 7 points. At Stanford the conclusion was drawn that measurable home environment contributes about 17 per cent. to the variability of children in such communities as were studied; that superior foster home environment had raised the IQ's of the subject about 7 points, and that extremely superior or inferior environment might alter the IQ by as much as 20 points. It was further concluded that since the correlations established between parents and offspring in the control group show good agreement with theoretical expectation on a genetic interpretation, heredity probably accounts for most of the variability in the children's ability.

The discrepancies between the Stanford and Chicago studies are not as serious as they might appear at first glance, but it would be highly desirable to get them explained and ironed out. We may hope that Miss Leahy's extended research on foster children, now in progress in Minnesota, may resolve the difficulties.

SPECIAL STUDY OF DATA ON FAMILY RESEMBLANCE

Only a few studies, other than the Stanford investigation just summarized, have attempted to reach a numerical estimate of the proportional contributions of nature and nurture to mental differences, or to explain the mechanics of the transmission of mental traits.

Willoughby (1927) used Fisher's method (1918) of estimating the relative contributions of nature and nur-

ture to physical traits on the basis of the discrepancies between parental and fraternal correlations. The estimate yielded relative contributions of about 50-50, but it is doubtful whether Fisher's method is at all applicable in Willoughby's study of mental traits, since environment is assumed by Fisher to operate by chance to *lower* correlations, instead of systematically to raise them.

Ruth Sherman Tolman and the writer (1932) compared the mental resemblance of sibling pairs who were very similar in physical appearance with that of sibling pairs who were less similar in physical appearance. The problem was approached under the supposition that chromosome linkage might be demonstrated between physical and mental composites due to many genes. Since siblings very similar in one composite would theoretically have an unusually high number of chromosomes in common, they should tend toward high resemblance in any other traits whose genes were located in some of the same chromosomes. The results of the Burks-Tolman investigation were negative. It is possible that the criterion for selection of the sibling groups was not sufficiently rigorous. Further work along this track might yield results of considerable interest.

RECAPITULATION

Out of the evidence which has been accumulating since Galton raised the nature-nurture problem from the level of mere speculation, what do we now know or feel fairly sure of? It seems clear:

That mental abilities in man cluster in family lines, and in racial and social groups.

That one can predict the intelligence of relatives of individuals of known intelligence with known accuracy or inaccuracy; and that even if heredity solely accounts for mental variability, children of the same family will differ, because they have differing heredity.

That complete deprivation of schooling probably has a depressing effect on the IQ, but that ordinary differ-

ences in education probably exert little influence upon the IQ.

That intellectual differences found between urban and rural children are probably due in large part to selective drainage of the higher levels of ability to the cities.

That measurable home environment probably contributes about 17 per cent. to children's mental variability, and that the major share of the remaining variability is probably due to heredity. It is likely that home environment at its best or worst can enhance or depress the IQ by about 20 IQ points.

That a cumulative Mendelian factor mechanism probably accounts for the transmission of intelligence, since all known heredity traits, including the mental traits, fit the Mendelian hypothesis, and environment demonstrably can not account for the correlations.

NEEDED EVIDENCE

The foregoing recapitulation, which most students of the field would agree could not be greatly extended through any facts at present available, contains the vital and central conclusion that mental ability is chiefly inherited. There are nevertheless ramifications of the problem which are in need of greater clarification. Because the number of contributors to this symposium is so small, the present contributor has taken soundings from other interested people. We may recall that Cattell has expressed the conviction that every scientist's best work is based on the original ideas he had before the age of twenty-five. Accordingly, several now or recently from the graduate student group as well as those known for their work in the field have been asked for suggestions.

A number of those consulted seem to have the view that like a chemist who controls the environment of his compound with respect to pressure, temperature, surrounding atmosphere and surface of container, we too must control our experiments, and not look to much help

from the sticky fly paper technique of catching up data which simply happen to be there.

The suggestions group themselves about environmental influences—including home, schooling, community and general life experiences—and heredity—including race and genetic mechanism. Projects contemplated by the writer are included with those proposed by others.

Home Environment

Alice M. Leahy, Institute of Child Welfare, University of Minnesota: Would like to see the discrepancies resolved between Stanford and Chicago studies of foster children. Is working now upon a carefully planned investigation which promises to do so.

Harold E. Jones, Institute of Child Welfare, University of California: Proposes the establishment, in cottage communities associated with two comparable factories under single management, experimental and control groups for determining the influences of environmental variables such as feeding, discipline, nursery school attendance, cultural advantages in the home, etc.

Frank Lorimer, Washington, D. C.: Wishes to test the possible differential effect of home environment upon different levels of ability; also to test the facilitation of mental growth through intensive instruction in language in earliest childhood.

Barbara Burks, Institute of Child Welfare, University of California: Plans if the opportunity offers, to establish an institution in which infants relinquished by their mothers may be reared for a term of years subject to various methods of conditioning.

Lewis M. Terman, Stanford University: Still subscribes to what he has termed *the experiment*—the selection of a large group of sibling pairs from an unfavored environment, one member of each pair to remain in the original milieu, and the other to be reared in a superior environment.

Schooling Effects

Bronson Price, graduate student, Stanford University: Suggests the experiment of introducing schools into poor mountain white communities having no educational facilities, and measuring the mental traits of children before and after attendance.

Community Effects

Sidney Adams, graduate student, University of California: Would like to test the hypothesis that the intellectual level of a community affects the intelligence of all its members, by evaluating the cumulative effect of selective emigration upon those who remain.

Combined Life Experiences

Harold Carter, Institute of Child Welfare, University of California: Plans to devote a great deal of time to the study of adult identical twins separated for varying periods of years.

Barbara Burks: Wishes to study overlapping effects of various environmental influences,—to ascertain, for example, whether the separate contributions of nursery school, home, stimulation through reading to a child or teaching him number work, etc., are additive, partly additive, or mainly overlapping.

Race

An inter-racial orphanage was proposed several years ago by the Social Science Research Council.

Genetics of Mental Heredity

Herbert S. Conrad, Institute of Child Welfare, University of California: Will be satisfied with nothing less than a three-generation study of the transmission of mental traits.

Barbara Burks: By establishing the regression of offspring on parents at different ability levels, will approach the problem of dominance and recessiveness in mental traits. Would like to continue approach to genetic linkage as described on p. 216 ff.

Robert Tryon, University of California: Through experiments in breeding rats on the basis of maze-running ability, hopes to illuminate the general problem of the transmission of mental traits. Wishes to obtain homozygous strains of rats, and then to experiment with specific environmental influences and with cross matings.

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PALAMEDES¹

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THE advance of science, which formerly inspired mankind with confidence, has in recent years resulted in diffidence and even despair. In ancient times Archimedes could boast, "Give me where to stand and I will move the earth"; at the beginning of the modern era Francis Bacon took the whole world for his province. Newton's discoveries (so Halley said) raised man to the level of the gods; and subsequent discoveries raised him above divinity. Laplace found God an unnecessary hypothesis in astronomy, Lyell in geology, Darwin in biology. Thus the drama of Eden was reenacted on a larger stage and with the rôles reversed: God was banished from both heaven and earth, while Huxley remarked in a satanic and audible aside that man had created God in his own image, and Swinburne sang like an archangel:

Glory to Man in the highest! for Man is the master of things.

There was, toward the end of the nineteenth century, a general impression that through science man had indeed conquered the external world and laid down its laws once and for all. There seemed nothing more to do but to turn science inward on ourselves; and that was what Galton proposed in his program of eugenics. The human species had outstripped all others in the evolutionary race; could it not go still farther? Could not men

rise on stepping stones
Of their dead selves to higher things†

Somehow this boasting is out of tune with the temper of to-day. The external world appears much more complicated than it did in the last century; and the founda-

¹ Paper read at the symposium of the American Society of Naturalists on "Heredity and Environment in Man," Atlantic City, December 30, 1932. Copyright, 1933, by Alexander Weinstein.

tions on which it rested so firmly are everywhere giving way beneath it. No longer do we stand firmly on the earth and raise our heads proudly among the stars; rather we totter precariously on shifting sands and peer tremulously into the darkness. Whether or not one shares this attitude, one can not help realizing its prevalence.

To the loss of self-confidence there have been three different reactions. One is an attempt to turn away from science and go back to a prescientific mode of living. This has been the program of the romanticists and the advocates of a simple life; of Samuel Butler in his proposal to abolish machines and of his numerous successors who cry out against the machine age of to-day. The suggestion has even been put forward that for a time we suspend research and take a holiday from science. This revolt against civilization has often been inspired by a love of nature, and there is something in it that would appeal to a society of naturalists; but on the whole it is of course an avoidance of the problem rather than a solution. A second proposal is to continue the scientific transformation of the environment to the utmost and so make the world a better place to live in. This is the solution most generally advocated, particularly by scientists, who have already done so much to transform the world. But even those who realize its necessity are beginning to doubt whether such a transformation is a sufficient solution of the problem. The human race, or at any rate a large part of it, seems incapable of coping with the complexities of the civilization which it has itself devised; how will it control a civilization still more complicated? There seems to be a real need for an increase in man's inborn capacities. Thus a third proposal is to adopt the program of eugenics; and even to those who are not entirely convinced of its necessity, the improvement of future generations has a moral and an esthetic appeal.

If we are to make a better breed of men, we must first decide what we mean by "better" and by "good." Thus

scientists found themselves confronted with a problem in ethics; this, too, at a time when they had been congratulating themselves on having finally and irrevocably banished from their outlook the moral considerations so common in ancient and medieval science. Moreover, the scientist could not, like the philosopher, approach the problem in leisurely fashion; he had to take it in his stride and solve it quickly as a mere preliminary to a problem of his own. A way out of the difficulty seemed to be indicated by a suggestion of Galton's: could we not discover the direction in which evolution is tending and accelerate its progress?² It appeared obvious that as evolution had transformed amoeba into man, so it would transform man into superman. To many it seemed obvious also that within the human species the victor in the evolutionary struggle was the white race: "Better fifty years of Europe than a cycle of Cathay." And within the white race the most successful were obviously the upper classes. If we selected from these we should be helping evolution along—giving the inevitable a shove in the right direction.

But the inevitable refused to be shoved. The upper classes, far from supplanting the others, were themselves found to be dying out because of their low birth rate. It was even said that the very existence of the white race was threatened by the prolificity of others; and while politicians spoke of the yellow peril there was talk in biological circles of the rising tide of color. The logical conclusion from all this was of course that the white race and particularly its upper classes are not the fittest in an evolutionary sense; but this conclusion was distasteful to the eugenists, who were themselves largely upper-class whites. They decided instead that the law of the survival of the fittest was being broken and began to cry aloud for its enforcement: they wanted conditions made such that the existence of the upper classes would be guaranteed.

² Francis Galton, "Inquiries into Human Faculty," London, 1883, p. 304.

Whatever we may think of such a procedure, its justification is no longer in evolution but in our own preferences; and if our preferences are to have any objective validity, we must be certain first that race and class differences are genetic, and second that the qualities we prefer are really superior.

The hypothesis that all race and class differences are genetic is often based on views attributed to Galton; it is commonly believed that as a result of his researches he came to the conclusion that nature prevails enormously over nurture. Most biologists would probably consider it a travesty to say that what Galton proved was that differences in heredity are more potent than differences in environment when the differences in environment are not too great; but that is the meaning of Galton's own summaries of his results. "The evidence was overwhelming that the power of nature was far stronger than that of nurture, when the natures of the persons compared were not exceedingly different." "There is no escape from the conclusion that nature prevails enormously over nurture when the differences of nurture do not exceed what is commonly to be found among persons of the same rank of society and in the same country."³ Obviously this does not mean that environmental differences between races or classes can be neglected.

Galton himself believed that the environment affects many traits:

Different aspects of the multifarious character of man respond to different calls from without, so that the same individual, and, much more, the same race, may behave very differently at different epochs. There may have been no fundamental change of character, but a different phase or mood of it may have been evoked by special circumstances, or those persons in whom that mood is naturally dominant may through some accident have the opportunity of acting for the time as representatives of the race. . . . Sudden eras of great intellectual progress can not be due to any alteration in the natural faculties of the race, because there has not been time for that, but to their being directed in productive channels.⁴

³ Francis Galton, "Memories of My Life," London, 1908, pp. 294-295. "Inquiries into Human Faculty," p. 241.

⁴ Francis Galton, "Inquiries into Human Faculty," pp. 178, 179.

It is often said that savages differ fundamentally from civilized peoples in being conservative and unenterprising. Even this difference Galton explained environmentally. To a civilized man, he said, change is easy because of the abundance of his material resources; whereas to a savage, whose resources are small, change is difficult so that "he is perforce taught to be conservative, his ideas are fixed, and he becomes scandalized even at the suggestion of change."⁵

Differences within the same race and social class were also attributed by Galton to environment. He thought that it was "owing to the favourable conditions of their early training, that an unusually large proportion of the sons of the most gifted men of science become distinguished in the same career."⁶ He ascribed the choice of a scientific or of a church career to the influence of the mother in early childhood. Galton held such strong views on the persistence of childhood impressions that it is surprising he has not been adopted by the Freudians.

The furniture of a man's mind [he wrote] chiefly consists of his recollections and the bonds that unite them. As all this is the fruit of experience, it must differ greatly in different minds according to their individual experiences. I have endeavoured to take stock of my own mental furniture in the way described in the next chapter, in which it will be seen how large a part consists of childish recollections, testifying to the permanent effect of many of the results of early education. The same fact has been strongly brought out by the replies from correspondents whom I had questioned on their mental imagery. It was frequently stated that the mental image invariably evoked by certain words was some event of childish experience or fancy. . . .

Our abstract ideas being mostly drawn from external experiences, their character also must depend upon the events of our individual histories. . . . The character of our abstract ideas, therefore, depends, to a considerable degree, on our nurture.⁷

I have quoted Galton in some detail because he is generally misrepresented, and because his views, while not necessarily correct, were based on a careful and penetrating examination of much evidence. Occasion-

⁵ *Ibid.*, p. 180.

⁶ Francis Galton, "Hereditary Genius," p. 197, London, 1869.

⁷ Francis Galton, "Inquiries into Human Faculty," pp. 182-183.

ally, it is true, his words can be interpreted to mean that the power of heredity is overwhelming and the influence of environment negligible; but only, I think, because he has neglected to say explicitly that he is speaking not of environment in general, but of "Nurture within the limited range that I have been careful to assign to the latter."⁸

Galton's successors, the biometricians, have found that the extent to which a character is correlated with the same character in related individuals is often greater than the extent to which it is correlated with some aspect of the environment. These correlations however do not necessarily measure the strength of heredity and of environment respectively. On the one hand, the similarity of related individuals may be due to environment, as in language or in infectious or nutritional diseases. On the other hand, the fact that a character is independent of some aspects of the environment does not demonstrate its independence of other aspects. Moreover, the correlations worked out were not very numerous, certainly not numerous enough to warrant a generalization as to the stability of all characters.

The last difficulty was apparently met by Mendelian studies; for of the numerous traits found to be inherited in Mendelian fashion, relatively few were influenced by environment. The traits studied however, although numerous, are not representative: they are used as indicators of the presence of genes; and traits that vary with environment, being poor indicators, are discarded as bad characters. It is because geneticists have concerned themselves mostly with constant traits that the traits they have studied have turned out to be constant. This is not the first time that scientists have mistaken limitations arbitrarily imposed by themselves for laws of nature.

Despite the unrepresentative character of the material, many Mendelian traits have been found which are

⁸ *Ibid.*, p. 235.

affected by environment as much as by heredity, or even more; a result borne out by the differences observed by Professor Muller and Professor Newman in identical twins reared apart. Physiologists also have discovered many differences in growth, development and behavior produced by small and hitherto unsuspected differences in food, air or sunlight. Professor Pavlov found his experimental animals sensitive to minute stimuli. The "slightest movements [of the experimenter]—blinking of the eyelids or movement of the eyes, posture, respiration and so on—all acted as stimuli which, falling upon the dog, were sufficient to vitiate the experiments by making exact interpretation of the results extremely difficult . . . the experimenter . . . had to be stationed outside the room in which the dog was placed, and even this precaution proved unsuccessful in laboratories not specially designed for the study of these particular reflexes. . . . To get over all these disturbing factors a special laboratory was built. . . ."

Such precautions are taken as a matter of course in experiments with animals. Yet, although man's behavior is known to be more modifiable than that of other species, it is often assumed to be unaffected by the conditions under which different races or classes live. A prominent psychologist who tried to test the ingenuity of cats by using hunger as a stimulus was criticized for basing conclusions about cats in general on hungry specimens. Many eugenists however write as if the educability of poor children is not influenced by lack of food.

There is a wide-spread belief that races differ greatly in mental capacity and that the differences are genetic. Both of these propositions are open to serious doubt. It is true that peoples may be very dissimilar in what they attend to or how they express themselves or how they behave; but when the thought processes underlying these diverse activities are examined, they are found to be essentially alike. The general impression that savages

* I. P. Pavlov, "Conditioned Reflexes," Oxford, 1927, p. 20.

are unreasonable is due to a lack of acquaintance with their motives; for the civilized men who visit savages or live among them are generally ignorant of their language and customs. It is notorious that travelers often give very erroneous impressions of the customs and ideas even of civilized nations closely related to their own.

Even knowledge, however, is not a guaranty against prejudice; and it is well to remember the words written by Herodotus some 2,400 years ago:

If it were proposed to all nations to choose which seemed best of all customs, each, after examination made, would place its own first; so well is each persuaded that its own are by far the best.¹⁰

Galton, despite his realization of the importance of environment, nevertheless attributed to heredity many racial differences in mentality; and he argued for the superiority of the white over savage races. He adduced however no cogent evidence for these views, and sometimes his statements are curiously naive. "It is seldom," he wrote, "that we hear of a white traveller meeting with a black chief whom he feels to be the better man."¹¹ Travelers are not noted for any tendency to admit their inferiority to the people among whom they travel, and they would scarcely be likely to make an exception in favor of savages. Moreover, a traveler who is not a better man than the chiefs he visits might never return to tell any tale at all.

So similar are the essential thought processes of savages and civilized peoples that anthropological accounts often read like travesties of civilized customs; this in fact explains much of the charm of anthropological literature and why among laymen anthropology is one of the most popular sciences. And (to put the shoe on the other foot) satirists have never had any difficulty in rendering civilization ludicrous by merely describing without comment the actions of civilized nations.

¹⁰ Herodotus, Book 3, Chapter 38. The translation quoted is that of A. D. Godley (Loeb Classical Library).

¹¹ Francis Galton, "Hereditary Genius," p. 339.

It is sometimes said that savage races would have achieved civilization if they had been capable of it. To substantiate such an argument, however, we should have to know why they did not achieve it and why we did; and this would require a far greater knowledge of the processes of cultural history than we possess. It is significant in this connection that even races often considered savage have made great cultural achievements (for example the Maya Indians, who developed a system of writing and a calendar superior to the European calendar of the time); that races once civilized have sunk to a lower stage of culture; and that the relative cultural standing of peoples has often changed in the course of history.

Comparisons have also been made between different races living together; for example, Negroes and whites in the United States. But these races are far from having the same environment. The housing and hygienic conditions and the educational facilities of the Negroes are much inferior to those of the whites; and there are besides the dissimilarities in tradition and the effects of race prejudice. To disregard environment in such comparisons is to shut one's eyes to plain facts. Some psychologists have attempted to devise mental tests that would measure only genetic traits; but attempts of this kind are futile, for no traits are purely genetic and mental traits in particular are susceptible to environmental influences. Professor Garth, who says that "his first investigations in the field of race psychology were accompanied by a silent conviction that he would find clear cut racial differences in mental processes," finally concluded, on the basis of his own work and that of others, that "we have never, with all our searching, found indisputable evidence for belief in mental differences which are essentially racial."¹²

It might be considered unlikely that evolution should have produced morphological racial differences without

¹² T. B. Garth, "Racial Psychology," New York, 1932, pp. vii, 24.

having produced psychological ones as well. It is true that the observed structural differences between races are not known to influence mentality; but surely there must be others that do. The argument however overlooks an essential distinction. Physical traits, if unimportant, will vary at random, unchecked by natural selection; and if important will vary by becoming adapted to different environments through natural selection. Mentality, however, being important, will not vary at random; in all environments it will tend, through natural selection, to become rational and hence uniform; for logic is logic from China to Peru and from Greenland's icy mountains to India's coral strand.

Even if evolution in the past had separated out diverse mental types, we should scarcely expect to find such differences characterizing large groups to-day; for throughout history there has been a continual mixture of races. In the oldest civilization known, that of Egypt, there was in the nineteenth century B. C. a royal edict against the coming of Negroes down the Nile. Wherever there is a prohibition there must be something to prohibit; and we can draw the conclusion that Negroes must have entered Egypt before the edict and probably after. In fact the very Pharaoh who issued the law boasted in another inscription that he had raided the lands of the Negroes and carried off their women.¹³ In the next oldest civilization known, that of Mesopotamia, we find at the opening of history at least two different races and probably more. The same is true of China, of Greece and of Italy. Early in her history Rome was invaded by barbarians from the north; and this continued with relatively slight intermissions until her fall. Even when there were no invasions, there was a continual importation of slaves from many other lands. In prehistoric times and throughout history migrations and wars, exploration, commerce and slavery have trans-

¹³ J. H. Breasted, "Records of Ancient Egypt," 1906, vol. 1, paragraphs 652, 658.

ported large numbers of people and often entire nations across all natural barriers. In view of facts like these, claims to racial purity on the part of nations are without genetic value. So-called national characteristics are much more likely to have their origin in environment or tradition than in hereditary constitution; they are perhaps still more likely to be inventions of historians.

The decline of nations is often attributed to an influx of alien blood. This argument has been applied particularly to Rome; but as a matter of fact much of Rome's greatness was due to foreigners. Her earliest writers were Greek slaves; her last great writer, Claudian, an Egyptian. Terence was a Carthaginian slave; Catullus and Livy came from Cisalpine Gaul; Seneca, Lucan, Martial from Spain. Of Roman poets perhaps the most typical were Virgil and Horace. Yet Virgil is not known to have had any Roman blood: he came from beyond the Po and was legally a Gaul until after he had attained manhood, when his province was enfranchised. Horace was not of Roman ancestry at all, as he was born in southern Italy, the son of an emancipated slave. When all roads lead to Rome, the able and ambitious tend to congregate there. Smaller communities also owe much to foreigners. Galton wrote: "It is very remarkable how large a proportion of the eminent men of all countries bear foreign names, and are the children of political refugees,—men well qualified to introduce a valuable strain of blood."¹⁴ It would, I think, be nearer the truth to ascribe the greatness of nations rather than their decline to foreigners.

These facts militate strongly against the oft-repeated assertion that race mixture in itself produces degeneration. Such a result would be expected on genetic theory only in special cases; race mixture in general should produce new combinations of genes, some doubtless worse but others even better than the original ones.

¹⁴ Francis Galton, "Hereditary Genius," p. 360.

Differences between classes are not so great as those between races; but they have been more minutely studied because they are of great practical importance. There are many persons, such as society leaders, editors of society columns, butlers, footmen, hotel clerks, and shopkeepers, who are as expert at distinguishing classes as anthropologists are at distinguishing races. Class distinctions, moreover, have been popularized by novelists. The aristocrat is proverbially tall, thin, and handsome. In England he used to have a dark complexion—a mark of his Norman blood; but this may now be altered by the popularity of the Nordic theory. He is well-dressed and has a characteristic manner; he is more sensitive than other people; he is interested in war and hunting. I am not aware that these distinctions have ever been studied statistically but they are generally supposed to be valid.

It used to be widely believed that a person of gentle blood could be recognized as such even if unaware of his ancestry and brought up among peasants and slaves. Herodotus tells such a story about Cyrus, king of the Persians; and many romances have turned on the same theme. I know of no experimental evidence that the distinctions are really genetic, unless we accept Hans Christian Andersen's account of the princess who was recognized as of royal blood because she was sensitive enough to feel a pea through a thickness of twenty mattresses and twenty featherbeds. There is, however, evidence against the genetic view of class distinctions. In Tudor England the costume of every social class, from nobility to laborers, was minutely regulated by law; and in colonial Virginia the lower classes were forbidden to dress too well, presumably lest they be mistaken for gentry. We know now that physical traits can be affected by nutrition and hygiene, and behavior by governesses and finishing schools; and our dictionaries define "breeding" as "the result of training." Nevertheless, the belief in the genetic nature of class distinctions has been

held even by scientific men; and a German gynecologist has asserted that while the sexual impulse is instinctive in women of the lower classes it is acquired in those of the upper classes.

Such beliefs have waned with the spread of democracy; but even in democratic countries it is still said that the upper classes are abler and more intelligent than the lower, that they alone know how to govern, that from their ranks come the great artists and writers and scientists. If a poor man becomes famous, the genealogists immediately begin to look for forgotten or illegitimate ancestors; a familiar instance is the attempt to trace Lincoln's pedigree to Chief Justice Marshall. What used to be considered romance now passes for eugenics.

It is of course true that the richer classes have contributed proportionally a greater number of eminent men than have the poor. But this is due in part at least to their better opportunities and there is no evidence that it is due to anything else. In a country like ours, where we hear equality spoken of so often, we come to believe that rich and poor have essentially the same chance of success. The belief is facilitated by the fact that rich and poor rarely know each other's life with any intimacy; hence the rich do not realize the obstacles confronting a poor man who would undertake an intellectual career, and the poor do not realize the extent to which these obstacles would disappear in an environment of wealth. The misunderstanding is increased by the fact that very few people of any class have an adequate comprehension of the psychology of creative intellectual work. There is a wide-spread notion that great ideas come as inspirations and that their coming can not be controlled or even predicted. It is true that great ideas often come at unexpected moments; but I doubt whether they ever come without preparation. They are the result of long intensive work, of complete absorption in a subject. Often the idea does not come until after the worker has rested from his labor; and this inactive period is probably the

source of the opinion that labor is unnecessary. Now, obviously, complete absorption in a subject is possible only where there is leisure; and this means that it is all but impossible for a really poor man, who has no leisure, to do great creative work. The sciences are now so complicated that years of preparation are required even to understand the important problems, and often years more of steady work for any solution.

In the arts the situation is often considered different. But although poets are born they must also be made, or at least they must not be unmade. Let me quote in this connection a passage from Sir Arthur Quiller-Couch:

What are the great poetical names of the last hundred years or so? Coleridge, Wordsworth, Byron, Shelley, Landor, Keats, Tennyson, Browning, Arnold, Morris, Rossetti, Swinburne—we may stop there. Of these, all but Keats, Browning, Rossetti were university men; and of these three Keats, who died young, cut off in his prime, was the only one not fairly well-to-do. It may seem a brutal thing to say, and it is a sad thing to say: but, as a matter of hard fact, the theory that poetical genius bloweth where it listeth, and equally in poor and rich, holds little truth. As a matter of hard fact, nine out of those twelve were university men; which means that somehow or other they procured the means to get the best education England can give. As a matter of hard fact, of the remaining three you know that Browning was well-to-do, and I challenge you that, if he had not been well-to-do, he would no more have attained to writing *Saul* or *The King and the Book* than Ruskin would have attained to writing *Modern Painters* if his father had not dealt prosperously in business. Rossetti had a small private income; and, moreover, he painted. There remains but Keats; whom Atropos slew young, as she slew John Clare in a madhouse, and James Thomson by the laudanum he took to drug disappointment. These are dreadful facts, but let us face them. It is—however dishonouring to us as a nation—certain that, by some fault in our commonwealth, the poor poet has not in these days, nor has had for two hundred years, a dog's chance. Believe me—and I have spent a great part of the last ten years in watching some 320 elementary schools—we may prate of democracy, but actually a poor child in England has little more hope than had the son of an Athenian slave to be emancipated into that intellectual freedom of which great writings are born.¹⁵

This is the statement not of a politician or a propagandist but of the King Edward VII professor of English literature at the University of Cambridge.

¹⁵ Arthur Quiller-Couch, "On the Art of Writing," New York, 1916, pp. 46-47.

There is a wide-spread notion that the really able will succeed, despite all obstacles. This involves a statistical fallacy: it might under certain circumstances be true if our lives were indefinitely long, but it does not apply to finite lifetimes. There is also a confusion between the fate of an idea and of its advocates. A true idea is perhaps bound to survive; but so far is this from applying to its discoverers or supporters that the best way to insure the survival of an idea is to undergo martyrdom for its sake.

Another common belief, expressed by Galton and others, is that the best strains of the lower classes have been drained into the upper classes. This involves two separate propositions: (1) that people have risen in the social scale, (2) that those who have risen are the most desirable. The first proposition is true, although it must not be forgotten that in the past the surmounting of class barriers has often been difficult or impossible. The second proposition is usually treated as self-evident; to most people it seems obvious that economic success depends upon intellectual ability. Thus a well-known eugenist said twenty years ago that good workingmen do not lose their jobs, that only incompetent men are unemployed. This showed a lack of historical knowledge, for economic conditions have in the past caused wide-spread unemployment. Only recently, however, another prominent eugenist said that the present depression would have a good effect by weeding out the unfit.

Now in spite of the fact that in times like the present the economic system picks largely at random, it may well be that at other times the people who became rich were selected to some extent at least on the basis of genetic traits. But are these traits the desirable ones? They have not always been so considered. The aristocratic classes have always looked down on those who become wealthy through their own efforts. The Catholic Church, in the middle ages and during the reformation, objected

to the ordinary practises involved in business, such as lending money at interest; so, too, did Luther and other Protestant leaders. Modern liberals and radicals echo these objections, or carry them to their logical conclusion as in Proudhon's statement that "property is theft." Such views have received some support from Francis Galton, who in his "*Memories*" expressed a fear that marriages of English peers with daughters of millionaires might result in a lowering of the standards of commercial probity of the House of Lords in future generations. He might have added that many of the newer peerages have been conferred for success in business and many older peerages for services that can not be considered honest.

Francis Bacon, who was a man of wide experience and neither a visionary nor a radical, said that "there is rarely any rising but by a commixture of good and evil arts";¹⁶ a very mild opinion in comparison with that of Machiavelli, who had already explained how to rise without recourse to good arts at all. Machiavelli's ideas have been denounced ever since they were published but only, I think, because they gave away the game; they have never been refuted, and the "*Prince*" is still the best handbook for getting along in the world. Plato and Machiavelli are not usually bracketed together; but a passage in the "*Republic*" says that if there should ever be a perfectly just man, he would be crucified; while a perfectly unjust man would be successful in all his affairs, would be able to marry his children to whomever he pleased, and through his great riches could even propitiate heaven with splendid sacrifices, so that a better provision is made by both gods and men for the life of the unjust than for the life of the just. This passage reads as if it had been inspired by the fate of Socrates. The spectacle of Truth forever on the scaffold, Wrong

¹⁶ Francis Bacon, "*Essays. Of Nobility.*"

forever on the throne has brought about the widely prevalent sympathy with martyrdom, and has been a powerful influence in the spread of Christianity.

Some eugenists, admitting that success often results from non-conformity to accepted moral standards, have concluded that the standards should be changed. They have advocated selfishness and ruthlessness, have justified war, and have proposed to breed a race devoid of kindness and sympathy. Such a code is not debatable if it is put forward as good in itself.* If however the code is advocated as a means to ends which are generally considered good, then I think the arguments for it can be shown to be incorrect; if indeed a logical demonstration is still needed when the results of selfishness are evident all about us. Among eugenists the usual justification of selfish competition is that it has been responsible for evolutionary progress. But we are not bound by evolutionary precedents; and if there is anything that evolution teaches it is the possibility of new modes of behavior. Kindness and cooperation however are not new: they were developed (as Darwin pointed out) in the struggle for existence and helped man to survive and advance in prehistoric times. Some eugenists base their codes on the behavior of other species; but even if we agreed to imitate other organisms, we could still justify almost any code we pleased by selecting the proper model: we could go to the ant, or consider the lilies of the field.

Many who admit that success does not imply ethical conduct maintain nevertheless that it does imply intelligence. For example, Mr. Edgar Schuster, who was the first research fellow of the Galton Eugenics Laboratory, wrote:

It may be that the opportunities sometimes favour moral attributes of an anti-social kind, but they never favour a weak intellect; the predatory millionaire is perhaps ethically of the same standing as the burglar, but intellectually he far surpasses him.¹⁷

¹⁷ Edgar Schuster, "Eugenics." Baltimore, 1912, p. 225.

If the intelligent and the dishonest have both been rising to the upper classes, the resulting intermarriages between them must have produced a tendency for the two traits to be associated. This would explain many of the ills of society. But it is doubtful whether the tendency is general, because the amount of intelligence required for rising in the social scale is not necessarily great, and in many situations too much intelligence is an impediment. At a convention of business men some years ago an executive said that although every one laments the lack of able assistants, yet any man who turns out to be unusually intelligent is certain to be discharged because his superiors are afraid of being supplanted by him. In politics, too, intelligence (beyond a certain modicum) is a drawback. The best-known example is the American presidency. It has been often remarked that a first-rate man rarely obtains his party's nomination and that presidential candidates are usually selected from among the less able contenders. The intelligent are always looked at askance; it is no compliment to a man to say that he lives by his wits.

The aristocracy have often been undoubtedly inferior to many members of the lower classes. In ancient Rome many of the slaves had been men of high social standing in their original homes and were better educated than their masters; in fact the earliest Roman intelligentsia was composed very largely of Greek slaves. The medieval barons looked down on those who could read and write.

Class distinctions have in fact been based largely on irrational grounds. The social esteem in which traits are held is often in inverse proportion to their intrinsic or social value. Manual labor is almost universally despised; and this has been carried so far that even scientific experimentation and artistic activity have been considered degrading. Plato objected to the introduction

of mechanical methods into geometry;¹⁸ Archimedes considered his mechanical inventions unworthy of a scientist; and Lucian complained that even great sculptors were held in scorn.

You may turn out a Pheidias or a Polycleitus, to be sure, and create a number of wonderful works; but even so, though your art will be generally commended, no sensible observer will be found to wish himself like you; whatever your real qualities, you will always rank as a common craftsman who makes his living with his hands.¹⁹

Nor have class distinctions always been consistent. In ancient Greece actors might enjoy high social status, take an important part in the politics of their communities, and associate with kings. Shakespeare, as an actor, had in the eyes of the law the choice of becoming a retainer to a nobleman or being classed as a rogue and vagabond. Molière as an actor was not eligible for Christian burial. To-day actors may be knighted and actresses may marry noblemen or millionaires. When, in addition, we consider how much change in social position has resulted from causes beyond the control of single individuals (enslavement of prisoners of war, economic changes, etc.) it would be strange if class lines corresponded to genetic distinctions.

The strengthening of class barriers, which has been advocated by many eugenists, would therefore be eugenically useless and even harmful. For in so far as class distinctions are environmental, they make genetic differences harder to recognize; and in so far as they are genetic, the best qualities are not necessarily in the upper classes and there is even evidence that the reverse may be the case. It is a curious episode in the history of science that eugenists should have defended class distinctions; it is still more curious that they should have

¹⁸ But of course it is true that mechanical methods are not a substitute for logical proof.

¹⁹ Lucian, "The Vision," chapter 9. The translation is that of H. W. and F. G. Fowler, Oxford, 1905.

done so in the name of Galton. For Galton stated very emphatically the anti-eugenic effects of inherited wealth:

The sickly children of a wealthy family have a better chance of living and rearing offspring than the stalwart children of a poor one. As with the body, so with the mind. Poverty is more adverse to early marriages than is natural bad temper, or inferiority of intellect. In civilized society, money interposes her aegis between the law of natural selection and very many of its rightful victims. Scrofula and madness are naturalized among us by wealth; short-sightedness is becoming so. There seems no limit to the morbid tendencies of body or mind that might accumulate in a land where the law of primogeniture was general, and where riches were more esteemed than personal qualities.²⁰

The best form of civilization in respect to the improvement of the race, would be one in which society was not costly; where incomes were chiefly derived from professional sources, and not much through inheritance; where every lad had a chance of showing his abilities, and, if highly gifted, was enabled to achieve a first-class education and entrance into professional life, by the liberal help of the exhibitions and scholarships which he had gained in his early youth. . . .²¹

Darwin and Huxley realized that wealth might suspend or reverse natural selection. In Plato's eugenic commonwealth there was to be neither excessive riches nor poverty for ordinary citizens, and for superior individuals no private property at all. A still earlier eugenicist, Theognis of Megara, who was like Plato of aristocratic family, explained that poverty led to race-degeneration by forcing people to marry for money instead of for good genetic qualities.²²

Even professed egalitarians do not always realize how thorough an equality of opportunity is required as a basis for eugenics. It is not enough (in the words of Anatole France) to "forbid rich and poor alike to sleep under the bridges." There must be freedom of opportunity for all, and that too at all ages; because desirable qualities may manifest themselves at various periods of life. Even apart from any eugenic application, this is

²⁰ Francis Galton, "Hereditary Talent and Character," *Macmillan's Magazine*, vol. 12, p. 326 (1865).

²¹ Francis Galton, "Hereditary Genius," p. 362.

²² Theognis, "Elegies," ll. 183-196.

the only way in which man's genetic qualities can be studied; and geneticists should have the less hesitation in advocating it because they can shift the actual execution onto the shoulders of economists. All this however is only preliminary; there remains a much more difficult question: who is to recognize the superior individuals and select them for propagation?

This question has never been properly answered. Some have envisaged a system of eugenic committees elected democratically; but the failure of democracies to pick superior individuals would make this worse than useless. Many eugenists have turned to aristocracy. It should not be forgotten, however, that aristocracy has had its opportunity and failed more miserably than democracy. If we consider the politicians of the past whom we might be tempted to entrust with a scientific task, we can scarcely select one more enlightened than Francis Bacon. He was a scholar and a philosopher, an advocate of the experimental method and of the founding of scientific societies. In his time the greatest scientific advances were those connected with astronomy; yet he did not appreciate the work of Kepler or Galileo, who were his contemporaries, or even of Copernicus, who lived two generations earlier. William Harvey, who was his personal physician, said that Bacon wrote science like a lord chancellor; and I am afraid that any future lord chancellor is likely to write it in the same way.

The distrust of politicians has caused many to turn to non-political intellectuals. We have such a body in the electors of the Hall of Fame, who are entrusted with the task of drawing up a roll of honor of the country's most illustrious dead. Their decisions show the same preference for mediocrity: Willard Gibbs, generally accounted America's greatest scientist, has never been elected; Edgar Allan Poe, generally regarded as America's greatest man of letters, was not chosen until after many inferior writers.

But (you will say) it would be too much to expect all the electors to be well versed in thermodynamics or even in the principles of literary criticism; why not have experts in each field pick their own geniuses? That too has been tried. Perhaps the most famous body of experts among intellectuals is the Académie Française. I will refrain from reading you a list of the mediocrities whom it has honored with membership; but among those who have failed of election are Descartes, Pascal, Molière, LeSage, Diderot, Rousseau, Beaumarchais, André Chénier, Comte, Balzac, Stendhal, Dumas père, Gautier, Michelet, Flaubert, Maupassant, Daudet, Zola, Verlaine, Baudelaire, and many others. The Academy has excluded France's greatest philosophers, her greatest novelists, her greatest dramatist, and some of her greatest historians and poets. In artistic and literary competitions the prize is usually awarded to mediocre work; a fact so well known that it led eminent literary men to object to a recent proposal that the League of Nations establish prizes for the encouragement of young writers and artists.

The great scientific societies, which were originally founded to help the active prosecution of research, have come to confine themselves largely to honoring men who no longer do research. How far out of touch they are with important scientific developments is sometimes shown strikingly as in the rejection of Joule's paper by the Royal Society.

The record of universities is no better. After Plato's death the headship of the Academy fell not to Aristotle or to Heracleides of Pontus, but to mediocre men—Speusippus and Xenocrates. It is interesting in this connection that while Aristotle wrote, "Plato is dear but truth is dearer," Speusippus (who was Plato's nephew) spread the story that Plato was the son not of a mortal father but of Apollo. Modern universities were not hospitable to science when it began the advances that

have transformed civilization; that is why scientific societies were founded. "Neither Copernicus, nor Bacon, nor Boyle, nor Huygens, nor Leeuwenhoek, nor Swammerdam, nor Van Helmont, nor Kepler, nor Guericke, nor Fermat, nor Napier, nor Stevin, nor Pascal, nor Descartes ever taught at any school or university."²⁸ The universities have not even pretended to encourage literature. In Shakespeare's day, Oxford had so low an opinion of contemporary drama that the university authorities regularly paid visiting dramatic companies to depart without presenting any plays. When the third folio of Shakespeare's works was published, the Bodleian library at Oxford sold its only copy of the first folio, with other superfluous books, for £24, to repurchase it in 1905 for £3000. Shakespeare of course was not an Oxford man; but Oxford has had two great poets among her students. Of these Shelley was expelled; and Swinburne was quietly asked to leave, escaping expulsion only because his college feared to make itself ridiculous by a repetition of the Shelley episode.

To-day also unusual ability and originality fail to obtain recognition at universities: sometimes despite the most careful and conscientious attempts to discover them. This is illustrated by the wranglerships at Cambridge. Until recently all graduating students in mathematics were arranged in order of their success in the examinations, and it has been noticed that while the top man (Senior Wrangler) rarely became a great scientist the Second Wranglers have contributed many of Cambridge's greatest mathematicians and physicists. It is obvious that the examiners did not know how to test for great originality. Whatever the reason, it is a well-known fact that most teachers prefer students who merely follow in their footsteps to those who strike out new paths. It is rare for a university appointment to go to

²⁸ Preserved Smith, "History of Modern Culture," New York, 1930, pp. 343-344.

the logical candidate; usually the choice falls on some one less able. It is the fashion to blame this state of affairs on the autocratic interference of university presidents and trustees; but the great majority of selections are made by the faculty themselves and by the department concerned.

This regression toward inferiority is brought about by several causes. There is an economic factor. An associate or subordinate is often a competitor or a potential competitor for position or patronage, and to raise up an able rival involves an unnecessary risk. Sometimes the rivalry is personal. People wish to tower above their fellows; and if they can do it in no other way they can at least stand out because of the flatness of the surrounding country. This fact is very important because the great majority of people are lacking in altruism. A third cause is the difficulty of recognizing great achievements. We judge people by what intellectual standards we have; but the greatest men are those who transcend or overthrow old standards and set up new ones. They can not be judged until after their standards have been accepted; hence as long as there is intellectual advance the leaders of it will be misunderstood or not understood at all. A fourth cause is that great men may fail to accomplish anything commensurate with their ability. They attempt more tasks and greater tasks and on a grander scale. On the other hand, a mediocre man who merely follows in the footsteps of others is fairly certain to accomplish something and to be appreciated. Those who have undertaken great quests have often met with frustration; while many a man who has started out to seek his father's asses has found a kingdom.

The regularity and inevitability with which these forces work is not usually realized by those unfamiliar with the history of thought. Let us consider, for example, our own science of genetics, limiting ourselves, for obvious reasons, to geneticists of the past. Of these, none have

contributed more than Mendel, Darwin, Galton, and Bateson. Mendel discovered the laws of heredity when they were being sought by many of the world's greatest biologists: but no one paid any attention to his discovery. He corresponded with Naegeli, one of the foremost authorities on the subject, and sent him seeds; yet Naegeli, although he wrote a treatise on heredity, did not so much as mention Mendel's work. Mendel failed even to obtain a regular teaching position at a gymnasium, although his students have testified that he was an unusually good teacher.

Darwin did not distinguish himself at school, or at the Universities of Edinburgh and Cambridge, Britain's foremost medical and scientific institutions. Neither he nor Galton, who also went to Cambridge, took a degree with honors, which a moderately clever undergraduate can do; and according to Galton some of the ablest undergraduates of his time failed to take honors. It is an interesting speculation what would have happened to Darwin and Galton if they had not been rich, or to Bateson if his father had not been master of St. John's College at Cambridge; for the headmaster of Bateson's school wrote to his father: "It is very doubtful whether so vague and aimless a boy will profit by University life."²⁴ I think we may safely conclude that it takes a genius to catch a genius.

But, as I have said, genius is not enough.²⁵ What is needed is not only intelligence but goodness as well; and unfortunately there is no evidence that the two are positively correlated. In some situations, in fact, the correlation is negative: an intelligent judge is more likely to recognize ability in others, but for this very reason is more likely to be jealous.

²⁴ William Bateson, F.R.S., *Naturalist*, edited by Beatrice Bateson, Cambridge, 1928, p. 8.

²⁵ We need only recall, for example, Humphry Davy's opposition to Faraday's election to the Royal Society.

If those entrusted with a eugenic program are not good, it is better that they should not be intelligent either; for if they are intelligent they can recognize merit more easily and weed it out more certainly. Eugenics therefore is driven to a conclusion reached by all the great moralists: that the immediate need of the world is good men. Moralists, however, have made two great mistakes: they have attempted to base morality on theological sanctions, and they have tried to inculcate it by teaching. The first error has been often exposed; but the possibility of teaching virtue is still almost universally believed in, although I doubt whether history records a single instance of a real alteration in a person's moral quality. The constancy of moral traits may appear incompatible with the modifiability of other mental traits by environmental influences. I do not, however, claim that moral traits will remain constant under all circumstances whatsoever; it may be that some day goodness will be induced by vaccination or by injection. But the incentives to a moral life have not been essentially altered by social and economic changes in the past, and they are not likely to be altered by any changes that have been proposed for the future. If private property is abolished, men will still want power; if power is abolished (but that will be difficult) men will still want the approbation and esteem of their fellows. Even the greatest men have lapsed from honesty in order to enhance their reputations still further. So strong is the urge, that in order to be considered wise, men will act stupidly; and in order to be considered good, they will break every principle of morality.

Galton thought that eugenics might succeed if it became a religion; but the history of religious institutions reveals the same tendency to suppress merit as is found elsewhere. In the elections of popes, as in the nominations of American presidents, the leading candidate is rarely chosen. Religion has encouraged hypocrisy and

has persecuted, imprisoned, tortured and killed men of independent mind; and Galton himself pointed out that in this way the genetic qualities of the human race have been greatly impaired.

The religion advocated by Galton was not theology, but a sense of obligation to one's fellow-men combined with an emotional fervor. Such an attitude is found in some types of patriotism (though not in all). Yet even when a nation is in danger, politicians and military men continue their usual intrigues, often in accentuated form; while in ordinary individuals a national crisis such as war evokes the worst instincts.

It might be objected that in patriotism there is an irrational factor that would be absent in a scientific gospel. Let us therefore examine science itself, which often inspires a feeling of duty to mankind, including future generations. Despite this, scientists are not (except in the minds of innocent laymen) unselfish individuals; it is a very exceptional scientist who will not sacrifice the future of science to his own personal interests.

The betrayal of science is facilitated by the very faith it inspires; any setback it receives is regarded as temporary because of the wide-spread notion that in the end truth will prevail. A setback for eugenics, however, involving the extinction of valuable strains, might be irreparable. For this reason, and because it deals with man and what may be considered man's essential nature—his genetic constitution—eugenics might be expected to inspire a more intense devotion. The eugenic movement has in fact taken on a religious fervor, as Galton hoped; but I can not believe that he would have approved either the ideals or the methods advocated by most eugenicists. He stood for intellectual and moral enlightenment and for social justice; he looked upon eugenics as a method of doing away with the cruelty of natural selection. Many of those who speak in his name have advocated racial and religious prejudice and economic and political

oppression. They have sought to furnish a quasi-scientific foundation for political reaction, especially in Germany; and in the United States their pronouncements are not easily distinguishable from those of the Ku Klux Klan.

There remains Galton's other suggestion, already alluded to, that we ascertain whither evolution is going and hasten its progress. This is tantamount to a deification of evolution, at any rate if God is defined (in the words of Matthew Arnold) as "a force, not ourselves, making for righteousness." Evolutionary theology has sometimes been extended by the conjuring up of an anti-evolutionary devil, as in Tennyson's lines:

Evolution always striving toward some ideal good,
And Reversion always dragging Evolution in the mud.

All this is, however, mere confusion of thought. Properly speaking evolution does not necessarily make for good, nor is it going anywhere in particular. Selection will alter the human race, but the direction of the change depends on the social environment, and this is fashioned by ourselves. The evolutionary God, like so many others, has been created in man's own image.

This is the crux of the eugenic problem. There is almost no limit to what man might make of himself by altering his genetic constitution; but these possibilities can be realized only if he first becomes more altruistic and intelligent, and he is not sufficiently altruistic or intelligent to take this first step. Hence an attempt to put eugenics into practise would defeat its own ends. Any group likely to be entrusted with a eugenic program would be too unintelligent to recognize merit and too selfish to select any merit they might recognize. They would pick out for propagation individuals of mediocre ability and of less than mediocre morality; that is, those stupid enough to have no independent ideas and those dishonest enough to pretend to have none. Shelley was

deprived of his children by a court of law; a eugenic tribunal would have sterilized him. Barabbas is always preferred to Jesus, at any rate before Jesus is crucified.

Such reverse selection must have been going on throughout man's existence. It was remarked long ago that the race is not to the swift, nor the battle to the strong, neither yet bread to the wise, nor yet riches to men of understanding, nor yet favor to men of skill; and since the writer of those words said that there is nothing new under the sun, the survival of undesirable traits must have been an old phenomenon in his day. Even in prehistoric times, as Darwin pointed out, the bravest and most unselfish individuals of a tribe were less likely to survive and to leave offspring. All this would help to account for the fact that there has been, so far as we can tell, no improvement in the innate mental qualities of mankind throughout history, or for that matter since the end of the Cro-Magnon period. This lack of advance must be due also to the fact that man is no longer divided into small isolated groups; for the larger the population, the more difficult it is for a new trait to establish itself, especially if it is a trait like unselfishness.

There are even indications that a decline may have occurred: the Cro-Magnons were in many respects superior to the Neolithic men who succeeded them, and Galton rated the ancient Athenians above the modern English. It is possible that the conditions of civilization put too great a handicap on original and independent thinkers; that the best-adapted type is one with a less inquiring mentality, willing to abide by tradition and to follow routine without question.

The evidence for a genetic decline, particularly in the historic period, is not, however, conclusive. The selection making for a decline has not been rigorous, because of the large element played by chance in human affairs; and it has also been spurious to the extent that it has been based on non-genetic traits. Even where the quali-

ties selected have been genetic, they have not always been perpetuated; for (1) successful individuals are likely to come of infertile strains since in these the families are smaller and the children get a better start in life; (2) even if the successful individual is not infertile he often marries an heiress who, being an only child, comes of an infertile stock and leaves few offspring; (3) successful individuals (the upper classes) limit their families more than the unsuccessful (the lower classes).

Thus there has been a protective mechanism by which the self-seeking and ruthless have been isolated in an upper class and sterilized; while desirable individuals have been kept in the lower classes where they were more likely to reproduce. In this way good genes and combinations of genes have been preserved; but so they would have been if they had been propagated in tissue cultures. Man can not live by genes alone: life is the interaction of genes with the environment.

The extension of opportunity would allow good genotypes to express themselves; but it would not in itself solve the eugenic problem. The mechanism by which the self-seeking have been weeded out would no longer exist; and any attempt at deliberate eugenics would be more dangerous than under the present system since the superior individuals, being more easily recognizable, would be all the more certain to be eliminated. It would be tragic indeed if the human race should fail just when it had become aware of its possibilities, and in the very attempt to realize them.

There is perhaps one way out. Whatever may be true of the huge majority of mankind, there are a few whose instincts are good. If these banded together into a voluntary eugenic comradeship, they could ultimately supplant the others. Provided of course that the others would allow them. We may however be certain that such a project would not be tolerated and that those attempting it would be executed or sterilized. If it were

done at all, it would have to be done secretly; there would have to be a eugenic conspiracy. Probably some day such a conspiracy will be organized; but there is no guaranty that it will be the only one. Other groups are just as likely to form similar conspiracies to insure the perpetuation of their traits; and we can not predict that intellect and good will triumph over intellect and evil—or over stupidity if the odds are too great.

I have been asked who Palamedes was and why this paper is entitled with his name. The question is a good illustration of my theme. Palamedes was the greatest of the Greeks who fought before Troy. He was a scientist and an engineer. He devised a system of writing and methods of arithmetic computation. He traced the movements of the heavenly bodies and used them for marking the hours and for guidance in navigation. He fashioned weights and measures. He built the fortifications of the Greek camp. He even invented checkers and dice to keep the soldiers occupied when they were not fighting. Yet his name is unknown except to classical scholars, while every schoolboy is familiar with Agamemnon, who was only the titular commander-in-chief, and Achilles, who though brave and handsome was a good deal of a brute. And if any one is asked who was the wisest of the Greeks, he will say Nestor or Odysseus. Nestor, however, was wise only in the sense that being very old he could remember many things; and Odysseus was not wise at all but merely unscrupulous. Palamedes and Odysseus once measured wits against each other when Palamedes had been sent to persuade Odysseus to come to the war. Odysseus, unwilling to go, feigned madness and ploughed with an ox and an ass yoked together; but Palamedes placed Odysseus' infant son, Telemachus, before the plough, and Odysseus swerved aside, showing that he was not as mad as that. Odysseus accompanied the Greeks; but when he arrived before Troy he forged letters from the enemy, hid them in Pala-

medes' tent, and accused him of treason. The letters were found, and Palamedes was stoned to death.

It may be said that this is not a fair parable; that Palamedes displayed a want of intelligence in allowing such a plot to succeed. If he could foretell the movements of the stars, why could he not foresee the actions of Odysseus? The objection might be valid if the men had been older, for with experience we learn what our fellows are like; but when we are young each interprets others by himself, since he attributes to others the impulses he himself feels. Odysseus gauged others correctly not because he was wise but because he was evil and it so happened that in this respect most men resembled him. In a better world it is Palamedes who would have been the better judge of men. If we are to make a better world, we must breed people like Palamedes; the danger is that we shall breed people like Odysseus instead.

SOME METHODOLOGICAL ASPECTS OF HUMAN GENETICS¹

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THE most hopeful sign for the future of human genetics is the growing recognition for the need of a special methodology appropriate to the difficulties of studying a species in which mating can not be directly controlled by the investigator. The influence of nature and nurture can not be elucidated by experimental methods such as are employed in the laboratory, and special statistical difficulties arise from the small size of the family. To these three cardinal difficulties, which must be distinguished from merely practical obstacles to rapid progress in the study of human inheritance such as the protracted adolescence of the human species and the large number of human chromosomes, a fourth must be added, when we direct attention to genetic differences involved in differences of social behavior. This is that man is the most teachable of all animals, above all an animal with an unusually complex development of the investigatory reflexes, and the only animal in which an elaborate system of communication through the medium of speech exercises a predominant influence upon social relations. On this account human society is a unique biological phenomenon with unique laws of evolution.

Two types of inquiry into the phenomena of human inheritance may be distinguished. The first is genetic analysis in the traditional sense. By this I mean the recognition of discrete differences in organization (whether structural differences, metabolic differences or differences of behavior) attributable to single genes, and the study of the way in which such genes are related in the mechanism of linkage. The methodological problems involved in connection with manifestations of single gene

¹ Paper read at the symposium on "Heredity and Environment in Man," before the American Society of Naturalists, Atlantic City, December 30, 1932.

substitutions or the interaction of two mutant genes are easily solved on the assumption of random mating when somatic variability is negligible. The magnificent statistical work of Bernstein on the iso-agglutinins and more recent work by Snyder on "taste blindness" shows that the theory of random mating applies with a high degree of precision to the transmission of characteristics which are not readily recognizable. On that assumption the data provided by human pedigrees can be subjected to rigorous quantitative analysis to detect dominant or recessive single gene substitutions of the autosomal and X-borne type. Manifestations involving the interaction of two dominant or recessive genes can also be detected. Subject to one important qualification, which I shall mention later, rigorous genetic analysis on these lines is necessarily confined to gene substitutions which manifest themselves in almost any environment in which the individual can develop. The chief mathematical problems which await solution in this connection are (a) a further consideration of the consequences of assortative mating, (b) further development of the analysis of linkage along such lines as have been indicated in recent communications by Bernstein and by Wiener.

The development of the theory of inbreeding as applied both to autosomal and sex-lined genes offers the possibility of detecting the effect of genes whose manifestation is subject to a significant measure of somatic variability. The consanguinity test is the only one which enables us to distinguish between a single autosomal gene substitution and a double dominant condition when the manifestation of the gene is lethal. A systematic study of the constitution of the progeny of consanguineous unions promises possibilities for the detection of recessive genes which could not be recognized by the application of familial analysis of the kind to which I have referred. Large-scale investigations of this character have still to be undertaken. They might yield information concerning rare recessive genes subject to sufficient somatic variability as to defy familial analysis.

Apart from purely methodological issues, the most urgent practical problem in human genetics is the elucidation of differences which involve genotypes whose frequency in the population is of the same order of magnitude. With a sufficient number of such differences on record, human genetics will be provided with points of reference for assessing the linkage relations of rare genes. Serology offers an extremely fruitful field as the new M-N locus of Landsteiner and Levine emphasizes. The discovery of taste blindness for phenyl-thio-urea compounds shows that serology does not exhaust the field of possible discovery. It is already possible to test whether a rare gene is located upon one of four human chromosomes. We can now hopefully look forward to the time when the twenty-four human chromosomes of man will be mapped.

While the outlook for human genetics in this field of inquiry is vastly more hopeful than it seemed to be ten years ago, it would be a grave error to neglect the difficulties of rapid progress along a second line of inquiry. As distinct from genetic analysis in the traditional Mendelian sense, I refer to the practical problem of distinguishing between genetic differences and differences due to environment in populations where observed differences may be of both kinds. In this field the pitfalls are numerous. One noteworthy feature of a large body of inquiries into the problem of nature and nurture is the prevalence of a somewhat narrow conception of nurture itself. This is well illustrated by a recent inquiry into the genetic status of intelligence tests by Burks. Dr. Burks seeks to establish a measure of the respective contributions of heredity and environment to observed differences in intelligence test scores by correlations with various systems of assessment for home influence. Such methods of inquiry can legitimately be used to answer the question: How far do the class of environmental differences measured by such assessments contribute to differences in test scores? In the nature of the case they

only deal with a very small group of the infinite class of variables which constitute *environment* in the biological sense. By environment in the biological sense we mean all those agencies which contribute to the structure and behavior of an individual in contradistinction to the contribution made by the physical structure of the gametes at the moment of fertilization. In inquiries directed to elucidate environmental influences we are not entitled to make any *a priori* assumptions about the kind of environmental agencies with which we have to deal.

Two points are worthy of special emphasis in this context. The first is the danger of overlooking the importance of uterine environment, and the need for experimental research into factors of foetal nutrition and intra-uterine growth. It is only necessary to mention the recent work of Lionel Penrose upon "Mongolian Idiocy" to indicate the importance of the uterine environment, and to draw attention to the significance of order of birth and maternal age as indications of the possibility that differences in uterine environment constitute significant agencies. A second point is analogous. Maternal age and prenatal hygiene are important because there exists between the moment of fertilization and the moment of birth a highly significant period during which differences of physical environment can modify the course of structural development. Between birth and the age at which formal education begins there also exists a protracted and it may be highly significant period during which differences of social environment may affect the behavior of an individual. Hence the comparative constancy of a psychological index such as the Intelligence Quotient offers no presumption in favor of the view that it measures gene differences which would be manifest in almost any environment consistent with existence. While relying too largely on introspective methods and concepts of questionable validity the Freudian school have performed a service to human biology by focussing attention on the importance of the social environment

during the years when the basic patterns of conditioned behavior are established.

The relative importance of nature and nurture (using the latter term in the widest possible sense) raises issues of the greatest practical interest to the psychologist and sociologist in the field of social behavior. Stated as a problem in biology the relevant issue is: What kind of gene differences are associated with differences in the functional activity of the nervous system, and in what range of environment are they recognizable? To answer this question fully requires the closest association between the work of the geneticist and modern research upon the analysis of behavior. At present geneticists and nerve physiologists fraternize but seldom. In the past genetical discussion of questions touching upon their common field of interest has been very largely influenced by the teachings of the instinct psychologists of a bygone generation. For this reason many speculations upon social evolution prompted by the influence of the natural selection theory in its earlier phase must now be reexamined in the light of modern work on the central nervous system, as well as from the genetical standpoint.

The study of behavior in the lower animals reveals the existence of many simple reflex patterns which are consistent with a very wide range of external conditions. What has emerged preeminently from modern work such as that of Pavlov's school is that the relevant environment in which the behavior patterns of the higher animals arise is not a fixed and static, but a dynamic and ever-changing pattern of stimuli; that this ever-changing pattern of stimuli generates new patterns of conditioned reflexes and that the chronological no less than the spatial relations of the stimuli themselves are significant in producing such new patterns. This leaves the way open to the recognition that human society is a phenomenon *sui generis*, a phenomenon which owes its uniquely dynamic character to human inventiveness and the capacity

of the human species to capitalize the fruits of its tool-bearing pursuits for the use of future generations through the medium of speech and its substitutes. Whatever differences of inborn constitution distinguish individuals and groups of individuals living in different places at different periods, the outstanding biological peculiarity of man is the fact that an *infinite* of different behavior patterns is consistent with the same genetic basis.

The instinct psychology of the selectionist school encouraged the belief that the student of human genetics would be able to detect simple unit characters in the domain of social behavior. What we now know about the physiology of the nervous system does not encourage such a hope. It is all the more necessary to refine our methods for the evaluation of genetic and nurtural differences. For the study of variation in a population the method of correlation can provide a valuable mathematical instrument so long as the function of mathematics is clearly recognized. Statistical methods are always liable to be used without sufficient appreciation of the fact that mathematical technique is only valid in so far as the specific qualitative features of the problem are stated at the outset. The fruitful use of correlation technique depends upon a careful recognition of the framework of environmental differences and the framework of genetic differences within which a body of observations has been collected. The human family is a unit of physical and social environment as well as a unit of individuals with a community of genetic endowment. The use of correlation technique to ascribe additive contributions of nature and nurture to the observed variance within a population implicitly neglects the correlation between the framework of family environment and the framework of genealogical relationships. For reasons which I shall state at length on a later occasion I believe that the attempt to determine the respective contributions of nature and nurture to human variability is meaningless from the

biological standpoint. What we can ask is whether gene differences are associated with observed differences, and whether such gene differences are manifest throughout a wide or a restricted range of environment.

Thus the suggestion that the respective contributions of genetic and environmental factors may be evaluated by taking into account the theoretical deviation of the "somatic" from the "genetic" correlation with respect to dominance neglects the fact that the environment of sibs is more homogeneous than the environment of individuals belonging to different generations. In addition to this, the theoretical considerations advanced in support of this method do not cover cases where the contribution of sex-linked genes is significant. I shall refer to this later.

In discussing how differences of nature and nurture are related to observed differences in a population, we may distinguish between two general classes of problems. One is to detect gene differences which are manifest throughout the range of environment to which members of one and the same fraternity or family group are exposed, and the other is to detect gene differences which are manifest throughout the whole range of environment to which different families are exposed. Each of these may be further subdivided. Within the first group it is necessary to draw a distinction between members of the same family having the same birth rank (as when plural births occur) and (as is more usually the case) members of the same family and of different birth rank. Within the second group it is necessary to distinguish between members of different families belonging to the same occupational, cultural or geographical group and members of the different families belonging to different occupational, cultural or geographical groups. With these issues before us the following considerations suggest themselves.

(1) To detect gene differences which manifest themselves as differences between individuals of the same birth rank brought up together in the same family, a

comparison of the resemblance of identical and non-identical twins can yield valuable information. Such data must be interpreted with caution, because it is unjustifiable to assume that the environment of fraternal twins is as homogeneous as the environment of identical twins. The fact that members of an identical pair are more alike in other respects will tend to make their choice of an environment more similar than the environment chosen by members of a fraternal pair of twins. Using this method Holzinger comes to the conclusion that the mean difference in I. Q. between members of the same fraternity and birth rank is reduced by rather less than a half, when all gene differences within the family are eliminated. Tallman's figures yield the same result. Herrman, working in my laboratory, has examined five hundred pairs of London twins and arrives at the same estimate as Holzinger. In the light of these findings it is permissible to ask whether any significance can be attached to differences so far recorded for different occupational and racial groups.

(2) A comparison of the resemblance between ordinary sibs and fraternal twins may be used to detect whether birth rank differences limit the exhibition of gene differences. In connection with differences of intelligence the results hitherto obtained support the conclusion that fraternal twins are more alike than ordinary brothers and sisters. To use this method to the full advantage it is necessary to compare with fraternal twins both sibs separated by a small and by a large difference in age.

(3) For detecting gene differences which are recognizable throughout the whole range of nurture to which different families at the same social level or at different social levels are exposed, either of two methods may be used. We may standardize the genetic material by correlations of identical twins reared apart. This has not yet been done upon an adequate scale. A second method is to standardize the family environment of individuals who

are genetically dissimilar. In their admirable study upon intelligence test scores, Freeman, Holzinger and Mitchell, of Chicago University, have placed on record the correlation between adopted children and sibs of the families into which they have been adopted. They have also recorded correlations for intelligence quotients of sibs adopted into different homes. A very significant correlation between non-related children brought up in the same home emerged from this inquiry, and a conspicuous diminution of the correlation for ordinary sibs was obtained.

(4) One aspect of the use of correlation technique has been neglected in the past. My attention was originally drawn to it by a comparison of the correlation coefficients of twins of like and unlike sex. I refer to the effect of sex linkage upon the observed correlations between relatives. For the case of filial and fraternal correlations I have already given a preliminary treatment of this problem and have dealt with it more fully for remote relationships in a later communication. In this context it suffices to say that correlations between individuals of unlike sex may differ from the values hitherto predicted by theory either in virtue of differences of environment associated with sex or the contribution of sex-linked genes. A study of cousin correlations of the maternal and paternal type enables a distinction to be drawn.

My own conviction is that Freeman, Holzinger and Mitchell have made a most significant contribution to the methodology of correlation technique as applied to the nature-nurture problem. When used without proper regard for the limitations imposed by the way in which data are collected correlation methods yield conclusions which throw more light upon the social prejudices of the investigator than upon the problem of nature and nurture. When the experimental issues are clearly defined, correlation methods can provide us with the means of obtaining more exact scientific knowledge of gene differences than we possess at present.

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MUTATION AND ADAPTATION AS COMPONENT PARTS OF A UNIVERSAL PRINCIPLE:

II. THE AUTOCATALYSIS CURVE

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IN the previous section of this essay¹ we have traced out the rather surprising similarity of behavior of vapors and liquids, chemically reactive gaseous mixtures, plant species and certain other systems, on both sides of a critical zone, which was thought to be analogous to the state of mutation, or mutability, in plants as observed by DeVries. On both sides of this critical zone the systems appear to behave according to the Law of Adaptation. Our final picture then indicated that we have brief periods of mutation connected by long periods of adaptation, which DeVries had stated constituted a correct account of the history of plant species.

Such a series of discontinuities can only develop the quality of continuity if viewed as periodic systems. Periodicity is universally found and probably arises directly and indirectly from the manner in which the common forms of energy are propagated, principally from the sun. The form of the periodic cycles thus obtained appears to differ in one important respect. Whereas the periodic cycles involved in evolution are believed to manifest a progressively upward trend (or downward in case the change is regarded as degeneration) the pulsations representing heat, light and electricity are represented as moving parallel to one of the rectangular coordinates. However it is obvious that these rhythmical processes are represented in this way simply for convenience. By the introduction of a suitable constant any desired angular relation to the coordinates could be ob-

¹ AMER. NAT., Vol. LXVII, No. 709, p. 163.

tained. A similar result could be achieved by changing the coordinates. Thus for instance the swinging of a perfect pendulum may be represented so that the record of its oscillations would look something like the curve given in Fig. 1 in which C is the distance traveled and T is the time elapsed.

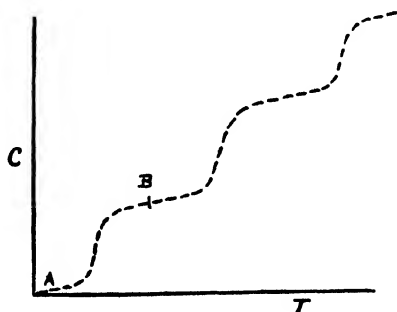


FIG. 1

If we take into account the fact that the two halves of a complete cycle have opposite signs, then one half of a complete oscillation would be represented by the line AB . The course of the whole process is recorded by passage from A to B and back again. Such a representation does not give us the history of the process that we are watching. For some purposes this would be of no consequence, but under other circumstances, as for instance when we wish to record time, the oscillations lose their identity and therefore their value as a means of measuring time unless some way of recording or designating the recurring rhythms or cycles is used.

It is important to note that many of the rhythmical cycles that have been previously mentioned are of this monotonously similar sort. Unless carefully observed each cycle is just like the other. Thus each day and night is for practical purposes alike, but it happens that for all of us certain days achieve a particular importance and so we find it necessary or desirable to mark each day, in order to give it its proper place in relation to the days

of other people. History, for the Greeks, lacked this chronological characteristic to a large extent.

But it is not the chronological aspect of our curve that we wish to consider. To any one familiar with the phenomena of autocatalysis it is at once evident that the portion *AB* of the curve in Fig. 1 represents the course of an autocatalyzed chemical reaction. In such reactions something occurs within the system that makes the reaction take place more and more easily. The typical form

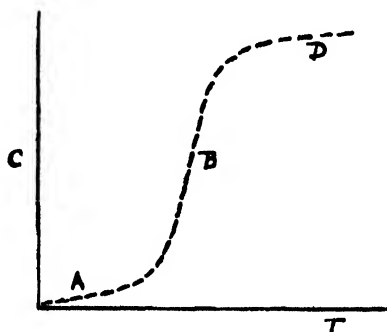


FIG. 2

of the curve is given in Fig. 2 and consists of a preliminary part *A*, known as the induction period, in which the change in *C* takes place very slowly. This is followed by a period of rapid change *B*, and then by a final period *D*, in which the change is slowly completed. It is evident that we are here dealing with phenomena that in some fundamental respect resemble those that we were discussing above. The fact that the phenomena in question are so different makes it difficult to see any relation, but the similarity of the curves is unmistakable. *A* represents an adaptive foreperiod, which in autocatalysis provides for the growth of the autocatalyst, *B* represents the period of mutation, or maximal activity of the autocatalyst, *i.e.*, a period of rapid change, and *D* is the period of completion and the foreperiod of the next cycle. In the physical processes that are represented by this curve we measure the ebb and flow of kinetic or potential energy

depending on our point of view; in the autocatalyzed processes we measure the rate of transformation of matter.

That such autocatalysis is probably a special case of a universal phenomenon has been recognized for some time and Wo. Ostwald invented the suggestive term autocatakinesis for the general phenomenon. T. B. Robertson has written a very interesting discussion of this topic under the heading "Growth as an Autocatalysis"² from which the following is taken:

This process (growth) does not take place with uniform velocity throughout life. . . . the process of growth is not one which undergoes uniform retardation, diminishing in velocity by a similar proportion per annum. On the contrary the growth of children, as also that of animals or plants, takes place in spurts, separated more or less distinctly from one another by periods of relatively languid growth. Thus, the rate of growth *in utero* during the first half of gestation is so slow that the weight of the human fetus is inappreciable in comparison with that of the mother during this period. This interval of slow growth is succeeded by the extraordinary rapid accretion of tissue which characterizes development during the months immediately prior to and succeeding delivery.

Towards the end of the first year of extrauterine life, however, there occurs a definite slackening of growth which is not an artifact. . . . This resting period or "plateau" in the curve of growth is succeeded by the relatively rapid growth of the third, fourth and fifth years of life. Another pause or slackening of growth succeeds this, to be followed by the energetic growth which accompanies adolescence. (*Loc. cit.*, p. 1-2.)

Similar growth curves have been obtained for every animal that has been studied. In seeking to interpret the meaning of this characteristic of the growth-process, we are immediately led to inquire whether any chemical processes of this particular type are known to occur elsewhere in the tissues of a growing organism.

As a matter of fact a number of processes of this character are familiar to the chemist. As examples we may cite the decomposition of cane sugar by boiling (neutral) water (Kullgren, *Zt. physik Chem.* 41, 407 (1902)), the decomposition of castor oil in the pulverized seeds of the castor bean (Constein, *Ergeb. d. Physiol.* 3, 194 (1904), the decomposition of methyl acetate by initially neutral water (W. Ostwald, *J. prakt. Chem.* (2), 28, 449 (1883)), the oxidation or "tarnishing" of metals and the oxidation of a variety of organic materials (J. W. Mellor, *Chemical Statics and Dynamics*). All these diverse processes have this feature in common, namely, that one of the products of the chemical change which is going on has the property of accelerating or "catalysing" the further progress of the change. . . . all of these processes are in a word "autocatalysed" or self-acceler-

² "The Chemical Basis of Growth and Senescence," 1923.

ated. The fact that the reaction has occurred is, up to a certain point, a favoring condition for its further occurrence. (*Loc. cit.*, p. 5-6.)

The fact that each growth cycle begins slowly and progressively increases in velocity until the moment of maximal growth-velocity is attained, at the center of the cycle, is sufficient in itself to show that the process of growth is autocatalysed. Up to the moment of maximal growth-velocity, the preceding growth affords a condition or series of conditions, favoring its continuance. The analogy extends much farther than this, however, for it has been found in a number of instances that in a cycle of growth the relationship of mass (or weight) to time is quantitatively identical with that which is displayed in autocatalytic reactions which have just been cited (*cf.* Chaps. II and III of this book for data). (*Loc. cit.*, p. 6.)

The phenomenon of growth has also been dealt with by Lotka. In an interesting chapter³ he has discussed the Law of Population Growth (the curve for which is similar in form to that for the Law of Autocatalysis which we are discussing) in terms of a general equation having a single variable. He showed that it has been applied quite accurately to the growth of the population of the United States (1790-1910) and to experimental⁴ populations such as *Drosophila* (fruit-flies) and to the growth of individual bacterial colonies. In connection with the behavior of the bacterial colony Lotka⁴ writes as follows:

A very particular interest attaches to this example, inasmuch as it forms, as it were, a connecting link between the law of growth of a population, and the law of growth of the individual. A colony of unicellular organisms, regarded as a whole, is analogous to the body of a multicellular organism. Or, to put the matter another way, a man, for example, may be regarded as a population of cells. We need not, therefore, be greatly surprised, if the growth of the multicellular organism should be found to follow a law similar to that exhibited by populations.

It is only fair to Lotka to state that he considers the analogy between the law of growth and the law of autocatalysis interesting, but he says it

must not be taken too seriously, inasmuch as in the one case the rate of growth is determined by ordinary chemical influence, in the other (organic growth) by a complicated combination of factors both of chemical and of physical character. Rather more to the point seems to be the suggestion made above in connection with the law of growth of bacterial colonies, that

³ Lotka, "Elements of Physical Biology," Chap. VII.

⁴ *Loc. cit.*, p. 69.

the body of the multicellular organism being a population of cells, it is not altogether surprising that it should be found to follow the law of growth of population.

The writer is under the impression that by a similar extension a chemical system is a "population" of molecules the growth of which is determined "by a complicated combination of factors both of chemical and of physical character." That is to say, the distinction pointed out by Lotka does not really exist. The important difference is the difference in "scale."

The above is sufficient to show that there is a fundamental similarity in these growth processes and those of an autocatalyzed chemical reaction. In some instances the process is complicated by the subsequent appearance of another cycle as in the growth studies of Robertson. If we assume that the retardation at the end of the first cycle is due to a depletion of one of the components, and if we attempt to determine which component it is, we must conclude that it is the autocatalyst that is depleted, because the cell group continues to live and the food supply is undiminished. Since there are three growth cycles in these animals we may assume that the same autocatalyst is developed three times in the life of the organism, or that each growth period is catalyzed by a different autocatalyst or autocatalytic system, and that as in physico-chemical systems we must know the history of our organism, as well as its life history, before we can predict its future behavior. A rat, for example, is bound by the same law as that which controls a gas, when considered in this way. Physiologists are already seeking for such a series of autocatalysts and a number of them have been found, but the details of these processes are still far from clear. Some of the factors involved are revealed by statistical studies concerning the growth of organs. The development of a maximum relative size for a given organ in a period of maximum in the growth and development of the organism as a whole, suggests a

regulative rôle for the organ in question. Since so far as is known the same organ never enters a maximum of this sort twice in the life of a normal individual it seems certain that different autocatalysts control the different maxima. If two or more autocatalysts are involved in giving rise to any given stage or change they may be regarded as constituting an autocatalytic system. In such a way we can view the process as controlled by a single variable, and we can for the moment leave out of consideration the fact that the variable itself often has a somewhat complicated history. In fact its formation may in turn be autocatalyzed at lower stages, and it may as a matter of fact show other effects in some of its stages or phases that render its chemical visualization and interpretation difficult, but this does not modify the fundamental simplicity of its action when considered *in toto*.

The idea of such autocatalyzed autocatalysts or cycles within cycles will become clearer in subsequent parts of this paper.

The life cycle: In the preceding section we have discussed the growth of a higher organism (typically mammals) as a succession of three autocatalysis cycles. In this section we wish to show how this cycle, or series of cycles, is followed by a reproductive cycle and then by extension we see other cycles. Finally, it becomes clear that a given organism may at a given moment be considered to be in several stages of rhythmic cycles having different "wave-lengths." The position of the organism in question upon an autocatalysis curve will then be determined by which autocatalytic process is being considered. It appears that the living organism in its parts and as a whole is involved in a large series of rhythmic cycles that are just as different in their "wave-lengths" as those emitted by the sun. We have the intra-atomic cycles and the molecular cycles in common with all matter. Then we have the physico-chemical intermolecular cycles involved in dialysis, adsorption, etc., by which the rhythmic quality of chemical interaction in the cell is pro-

duced. Then by small stages we come to the more obvious physiological rhythms, such as the flashing of a firefly and others, like peristalsis mentioned above. Then perhaps we come to the rhythmic rest periods produced by the alternation of day and night and winter and summer. Above these and with still longer "wave-lengths" come other rhythms in the life of the individual and the race that we now wish to discuss briefly.

These longer rhythms can best be discussed on the basis of examples. A carrot seedling, to take a simple example from the plant kingdom, is endowed with a sufficient store of food to elaborate and raise its "energy-binding" apparatus above ground. Here the light of the sun falls upon the chloroplasts of the carrot plant and solar energy is captured and stored, in part, in daughter chloroplasts called leucoplasts, principally as carbohydrates in the root. At first a considerable portion of the stored energy is used to extend the energy-binding machinery to make more chloroplasts. The storage of solar energy as starch by the leucoplasts is continued until the seasonal growth of the carrot is complete. The year's work is now done. The plant rests. When the carrot renews its growth the following season, it being normally a biennial plant, it appears to be possessed with a different "motive." Whereas in its first stage of growth it was an energy trap, a builder of a "savings account" in the form of a large tuberous root, it now seems to have "forgotten" all about this. Its chief interest now becomes that of an "unselfish self-sacrificing parent." The fine mature carrot spends itself in the reproductive effort of producing and of maturing its seed and then perishes. The seeds produced again repeat the cycle of the parent possessed of the same motive or "drive" at the corresponding stage of development. Each of these two stages of life in the carrot is controlled by its own autocatalyst. In the case of plants the nature of these autocatalysts is very little known, but it is obvious that they play the

same regulatory rôles as the hormones in animals. All plants do not utilize this rhythmic principle in the same way. The discussion of this simple example of the carrot is enough, however, to illustrate the application of the principle at this stage of organization of life.

A baby, like a carrot, is an "energy trap." Unlike the carrot it can not utilize the sun's rays as a source of growth and power, but must use solar energy that has been bound by plants. The destiny of the baby is determined to a considerable degree by the kind of bound energy offered to it and by the kind of a "trap" that it is. A proper ability to digest and convert food into more baby is a fundamental prerequisite. Presently under favorable conditions the baby, like the carrot, completes its growth and enters into the second cycle, or reproductive cycle of its physical existence. In the case of higher animals we know that this is coincident with the maturation of the sex organs and that the physiological aspects of its activities in this respect are controlled by internal secretions, that are carried back into the blood from these organs and that affect every physiological reaction and every psychological reaction of the organism in question, to a greater or less extent. In this case the autocatalytic curve is fully developed in recognizable form. We have the period of adaptation or growth of the autocatalysts in puberty; we have the period of maximal activity or mutation and then the period of completion at the end. But when we come to look at this long cycle more closely we see that it is accompanied by shorter seasonal cycles and physiological cycles so that in this field we can again find autocatalytic cycles of almost any "wave-length" that we desire, up to that of the maximum length of the reproductive cycle itself.

We thus see that the growth cycle and the reproductive cycle have the form of autocatalyzed processes and if nothing further were known about it, we could venture the suggestion that they are controlled and determined

by two series of physiological autocatalysts. As it happens some of these are already known to be internal secretions of certain tissues. It is worthy of note that the maxima for these autocatalytic processes come at different periods in the life-history of the individual. Thus man normally attains his growth and passes through his physical maximum of strength and power at or before his twenty-fifth year. By this time the growth autocatalysts have receded in importance, but the reproductive autocatalysts are just rising to their maximum. Meanwhile he has not yet attained intellectual maturity. The autocatalysts of intellectual power are somehow dependent upon those pertaining to growth and sexual ripeness, but the appearance of the maximum is delayed. In fact the best fruit of the mind is normally scarcely set before the fruit of the body has largely ripened and dropped off the tree, so to speak.

Finally, there is normally another growth that is also apparently secondarily dependent upon the completion of the functions of the autocatalysts of growth, of reproduction and of intellectual power. This we may call the growth of the spirit or the soul of man. The development of this indefinable quality is slow and appears to be dependent upon the successful completion of a normal maturity in the other processes enumerated. In this the development of the autocatalyst requires a longer period and the maximum usually appears late in the life of the individual. This is perhaps the most complex integration or coordination of the details of function and experience that is achieved in the life of man and up to the present our appraisal of the result of this development in any individual is largely intuitive. In fact it is almost impossible to discuss the matter in satisfactory terms.

Each of these autocatalytic waves is longer in duration than the preceding. The relations may be visualized from the diagram. It seems likely that the development

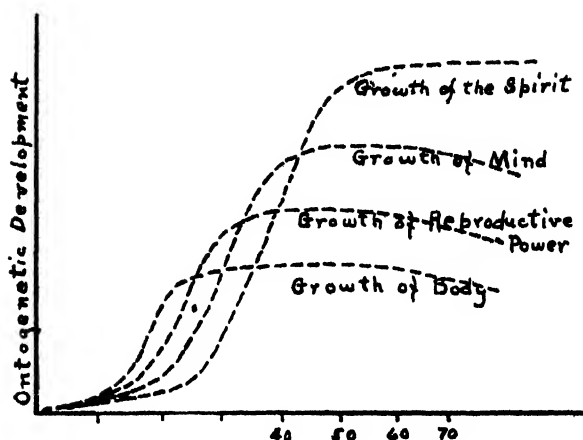


FIG. 3

of the autocatalysts for all of them takes place simultaneously, but in any case they come to maximum fruition at different times. At some stage each represents a prevailing preoccupation of the human race as a whole, and life is dominated by a different interest at each stage of the life of the individual. It is a well-known fact that failure to develop the autocatalysts of growth is physically fatal in the earlier stages of life. A baby that does not grow does not live long. Similarly failure to grow at later stages is also fatal to the proper growth and maturation of mind and soul. The problem of education and nurture is that of supplying the proper "building stones" or raw material to a system capable of utilizing them. These two agents then cooperate to build these intangible and almost inconceivable "catalysts" of the mind and soul. Here we have a profound chemistry about which we can say little or nothing at present.

Résumé: In the first section of this essay we reviewed a wide variety of phenomena in which two adaptable states are connected by a state of change or mutation. A graphical representation of these phenomena gives us an "S" curve that resembles the autocatalysis curve of

chemistry. The question at once arose as to whether this principle of rhythm or periodicity that is obtained by a synthesis of "adaptation" and "mutation" has any real relation to autocatalysis. For a restricted chemical or physical process as carried out in the laboratory the phenomena discussed in the first section and those of autocatalysis are subject to the same limitations. Usually not more than one cycle is observed. However, in the freer states of nature, particularly under the organizing influence of life processes, we found a succession of autocatalyzed processes. We found the form of the curve to be an indication of the existence of processes of this sort. Before attempting to get some general picture of what is taking place in these diverse systems, that makes them resemble each other, we ought to proceed somewhat farther in the next section with the description of this astonishing system of rhythmical and periodical phenomena.

SHORTER ARTICLES AND DISCUSSION

DICE ON GEOGRAPHIC VARIATION IN PEROMYSCUS

MAMMALOGISTS used to content themselves with studying, describing and classifying the skins and skulls of animals caught in the field. On such studies species and subspecies were defined and their geographic ranges mapped. But the up-to-date mammalogist is skeptical of the true significance of museum specimens. Before he accepts a particular specimen as typical of its species he wants to know how old it was when killed, at what time of year it was killed, and in what kind of an environment it had lived previously. Such data are seldom obtainable for field-caught specimens. So present-day mammalogists are taking to raising their own specimens under controlled environmental conditions and killing them for study when full growth and a known age have been attained.

A model study based on such material has recently been published by Dr. L. R. Dice.¹ It is of importance to students of evolution in general and of mammalian genetics in particular. That its significance may not be overlooked I venture to comment upon it.

The most widely distributed and commonest of North American mammals is probably the deer-mouse, *Peromyscus*. The well-known monograph of the genus by Osgood made some years ago has served as a basis for the subsequent intensive combined field and laboratory study made by Sumner and now being continued by Dice.

The particular study on which Dice now reports was made on the geographic variation of a single subspecies of *Peromyscus* which occurs throughout the corn-belt, ranging from Michigan and Ohio west and south across Indiana, Illinois and Missouri to Oklahoma and thence north to Manitoba. Dice collected animals from three widely separated localities within the range of this prairie subspecies, (1) Ann Arbor, Michigan, near the eastern limit of its range, (2) Alexander, Iowa, in the north-central portion of the range, and (3) Grafton, North Dakota, near its extreme northwestern limit. Young to the number of a hundred or more were reared from animals caught in each of these widely separated localities. All were kept under similar laboratory conditions and killed at similar ages. Weight, body

¹ L. R. Dice, *Occasional Papers, Mus. Zool., Univ. of Mich., No. 239, 1932.*

dimensions, skeletal dimensions, and pelage were carefully compared and recorded.

As regards the pelage "only relatively slight geographical differences in color" were observed, but as regards "the dimensions of several parts of the body and skeleton" significant differences are recorded.

Thus the North Dakota mice average about five grams heavier than the Ann Arbor mice; their body length is about four mm greater, their hind-foot about 1 mm longer and their ear-length more than 1 mm greater. Also femur length and skull length are greater in the North Dakota mice by about 1 mm. In short they are bigger animals in all dimensions.

These differences occur in animals bred in captivity under the same laboratory conditions and can accordingly only be referred to racial or genetic causes.

The Iowa mice, intermediate in geographic position are also intermediate in weight and body dimensions.

A glance at Dice's map of the range of the subspecies studied shows that the Mississippi river cuts squarely across the middle of it. Hence there can have been little interbreeding across this barrier since glacial times.

The only difference which can now be clearly discerned between animals occurring at opposite extremes of the range (after many centuries of separation) is one of general body size.

A prevalent view concerning the nature of size inheritance would regard it as due to the action of many independent genes having local effects. If so, we should expect that while some genes mutated in a plus direction, others would mutate in a minus direction. If so, some body dimensions should grow less while others grew larger. But this is not what Dice observes in the case of North Dakota as compared with Michigan stocks of *Peromyscus*. All bodily and skeletal dimensions are increased in unison. Dice does not emphasize this point, but I would call particular attention to it because it supports the conclusions reached in the studies which I have made of size inheritance in rabbits. There it is obvious that breed differences in weight, skull length, ear-length and other measurements increase or decrease in unison. This can be referred not to a multitude of independent genetic agencies, as the multiple factor hypothesis of size inheritance would seem to require, but to a single common agency. This common agency has been shown by studies of Gregory and myself to be in all probability developmental

rate of the embryo. What the genetic basis of *that* is remains yet to be demonstrated. It seems nevertheless to be an advance to be able to substitute a single unknown variable for many independent ones.

I would suggest that the only obvious evolutionary change which is occurring in *Peromyscus maniculatus bairdii*, as indicated by the intensive studies of Dice, is the development of slight differences in general body size at opposite extremes of its range. This difference is manifested in weight and various bodily and skeletal measurements, perhaps referable alike to a single agency, as in the case of the much greater size differences among domestic rabbits which all result naturally and of necessity from a difference in developmental rate of the embryo.

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"LEADEN," A RECENT COLOR MUTATION IN THE HOUSE MOUSE

WITH the increasingly large number of mammals now being raised for experimental purposes and kept under close laboratory observation, there is furnished a much greater opportunity for the detection of any visible change in the established characteristics of the stock animals. Such large groups of mammals tend to produce occasional variations not normal for the stock, but the detection and preservation of point mutations is still relatively rare.

Mutant characters in the house mouse, *Mus musculus*, are now being genetically established more often than in the past, due to the expansion of interest in the use of this valuable laboratory mammal, so that the species now has at least one known character on ten different chromosomes. The absence of known characters on the remaining ten chromosomes makes it imperative to test all new mutants as soon as they are genetically established in order to ascertain whether the character in question is independent and on a new chromosome or linked with one of the known chromosomal groups.

It is the purpose of this paper to report the origin of a new recessive mutant from a closely inbred strain of animals, to show the linkage tests and give a comparison of the first six generations of the mutant strain and the strain from which it arose.

A strain of mice known as "chocolate brown" has been inbred as a control stock in these laboratories for several years. Geneti-

cally the animals are non-agouti brown (aabb). In 1925, when the strain came under the writer's care, they were eleven generations inbred, having been produced from one pair of black animals which carried the recessive brown. The usual method of breeding has been to make brother to sister matings. When this was impossible a back-cross to the parent was made.

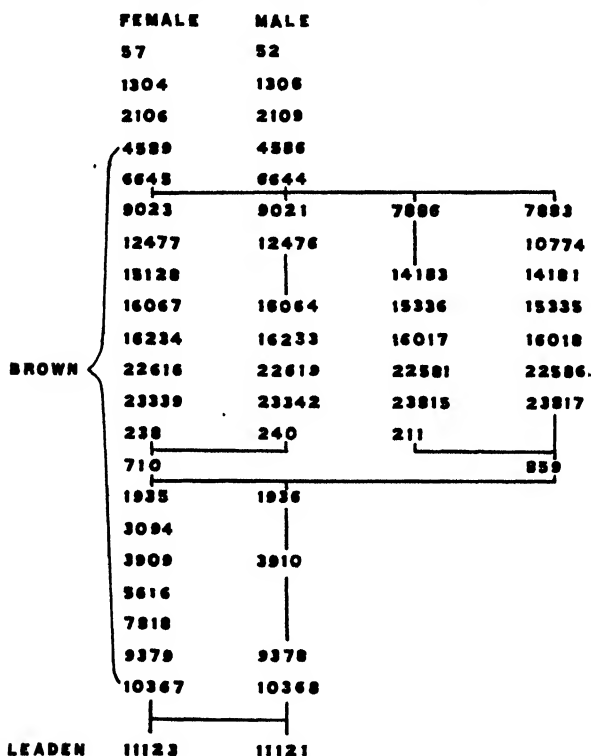


Fig. 1 is the pedigree chart which shows each recorded mating preceding the origin of the mutant character.

From this pedigree it will be seen that in the fifth generation two lines were established from two litters by the same pair of animals. In order to keep the strain viable these lines were united again to produce the fourteenth generation.

A great deal of difficulty was experienced in one particular line derived from this fourteenth generation. It was found necessary to back-cross to get the sixteenth generation and the male from this sixteenth generation was used to produce the 17th, 18th and 19th generations.

In the 21st generation a male and female were produced in the first litter from a mating between ♀ 10367 and ♂ 10368 which exhibited a marked dilution in the coat pigmentation. This dilution has proven to be independent of any known dilution factor and we have designated the character from gross appearance as "leaden," and given it the symbol "l."

Because of the phenotypical similarity between the leaden and blue dilution, as it appears in combination with brown, the progenitors (♀ 10367 and ♂ 10368) of the leaden animals were outcrossed to highly inbred blue-dilute (bbdd) animals. Male 10368 produced nine litters with 39 viable young, and female 10367 produced two litters with 9 viable young. All these offspring were chocolate brown in color. Although blue-dilution has never occurred in thousands of animals in the brown stock, these tests were made to eliminate the possibility of contamination in the particular animals giving rise to the leaden mutants.

Back-cross matings between leaden females and male 10368 produced seven brown and twelve leaden young. In back-cross matings between leaden males and brown females, heterozygous for leaden, six litters produced twenty-six brown and fourteen leaden young. These tests show the intense brown to be completely dominant to the leaden dilution.

Linkage tests were then begun by mating highly inbred dilute brown females to leaden males. Twenty-four litters of this cross produced 114 viable young, all of which were chocolate brown in color. This F_1 generation served as a final test to show that the new mutant color was not governed by the same gene as blue-dilution.

For linkage tests between dilute and leaden, the back-cross between F_1 animals and the double recessive was desirable, but it became evident, after several litters of F_2 animals had been weaned, that this test would be involved by the fact that double recessive animals were not phenotypically distinguishable from the homozygous leaden individuals which were heterozygous for blue-dilution or did not carry blue-dilution at all.

Of the animals tested, two males from the leaden and dilute leaden class in the F_2 generation were found to be homozygous for both blue-dilution and leaden. In the eighteen litters produced by mating these double recessive males to F_1 females, 154 young were raised and classified as follows:

Class	DL	dL	Dl + dl
Ratio exp.	1	1	2
Data	43	37	74
Expectation	38.5 ± 3.6	38.5 ± 3.6	77 ± 4.18

In order to substantiate these data a large F_2 generation was raised. According to expectation this F_2 generation should segregate in a ratio of 9:3:3:1 between chocolate, dilute, leaden and dilute-leaden. In this cross 853 animals were weaned from the 898 young born (102 litters) and were classified for coat color. Distribution of these classes is as follows:

Class	DL	dL	Dl + dl
Ratio exp.	9	3	4
Data	510	148	195
Expectation	479.7 ± 9.77	159.9 ± 7.68	213.2 ± 8.52

The excess of brown animals, a little over three times the probable error, is no doubt due to the fact that a postnatal selection was introduced in favor of the intense brown class. The litters produced by this cross were very large and since the young in the brown class were easily detected at six or seven days of age they were killed off to allow the dilute colored sibs a better chance for survival to weaning age. The data may be more accurately presented if only complete litters are used, that is, litters in which there was no mortality between birth and the time of classification for color, and in which no postnatal differential viability of young need be considered.

Sixty-eight of these undepleted litters produced 595 young with essentially the same distribution as was found for the total number of animals as classified in the last table.

Class	DL	dL	Dl + dl
Ratio exp.	9	3	4
Data	353	103	139
Expectation	333.9 ± 8.15	111.3 ± 6.41	148.4 ± 7.11

From the data of these tests there are no indications of linkage between the characters blue-dilution and leaden. In order to further substantiate these data similar tests were made with

short-ear, a character which has been reported to be very closely linked with dilution. Here also negative results were obtained.

The results of linkage tests with albinism, black, agouti, hairless, naked, dwarf, waltzing, dominant spotting and short-ear are tabulated in the following table:

Back-cross	XL	Xl	xL	xl
Black				
BbLl × bbll	69/70.5 ± 4.9	70	72	71
Agouti				
AaLl × aall	35/32.0 ± 3.3	38	26	29
Hairless				
Hrhr × hrhrll	24/22.5 ± 2.79	26	26	15
Naked				
NnLl × nnll	47/40.5 ± 3.72	37	39	39
Dominant-spotting				
WwLl × wwll	55/55.0 ± 4.33	51	56	58
Short-ear				
Sese × sesemm	45/51.2 ± 4.18	52	47	61
F₂				
Albinism			96	
CcLl × CcLl	182/189.9 ± 6.16	61/63.3 ± 4.85	84.4/ ± 5.37	
Hairless				
HrHrLl HrhrLl	59/58.5 ± 3.4	16/19.5 ± 2.67	19/19.5 ± 2.67	10/6.5 ± 1.66
Dwarf				
DwdwLl × DwdwLl	52/46.7 ± 3.0	16/15.6 ± 2.40	8/15.6 ± 2.40	7/5.2 ± 1.49
Waltzing				
VvLl × VvLl	72/79.8 ± 3.99	27/26.6 ± 3.14	34/26.6 ± 3.14	9/8.87 ± 1.95

In the tests with these nine characters the data show no indication of linkage, the segregation in each case being close to expectation for free assortment.

As is shown by the pedigree chart of the brown stock, before the mutation occurred the line of animals appeared to be getting progressively weaker. Only by an unusual amount of back-crossing was the line kept viable. It is of interest to note that following the germinal changes which manifest themselves in a recessive color character, the fertility and viability of the ani-

mals in the line was improved. This is shown in the breeding records of the two stocks when a comparison between the mutant and the last six generations of the brown stock is made.

Tabulated below are the data on age at first litter, number of young at first litter, percentage of young born dead, and percentage of young viable at weaning age (one month).

	No. litters	Ave. age 1st litter	Ave. no. young	Per cent. young born dead	Per cent. young weaned
Brown stock	680	103.4	5.83	9.12	42.4
Mutant stock	259	97.8	6.1	4.38	58.6

Since all the animals in both stocks have been kept under as uniform conditions as possible it would seem that these data are significant and show an improvement in the general physiological constitution of the mutant stock. This improvement over the original stock is not marked, but is uniformly better in all respects. Such improvement is not generally found when recessive mutant forms arise.

The material provided by this mutant is unique in that the records of inbreeding and long period of observation on the stock from which it arose are available for comparison. Arising from a stock which in general is above average in fertility and viability, the germinal readjustment has apparently eliminated weakening modifiers in the particular line from which it arose and has established a better physiological level than is found in the original stock as a whole.

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PROTOZOANS AND BEETLES

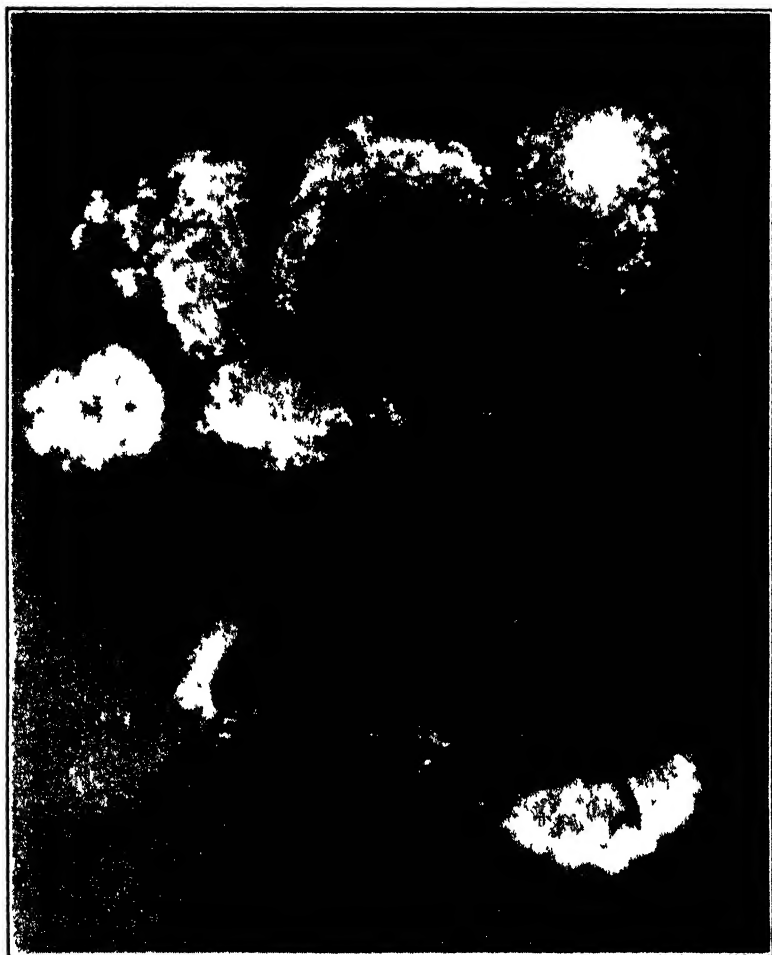
THE intimate relationship between certain insects and protozoans are well known to students of entomology and parasitology. The degree of the association may vary from true parasitism to harmless commensals with many intermediate stages. As examples of true protozoan parasites on insects *Nosema apis* and *Nosema bombycis* claim validity; the former causing a serious disease of honeybees, and the latter threatening the silk industry of France by causing pebrine in the silkworm larvae before the arrival of Pasteur. Less pronounced effects of parasitism in insects by protozoans are found among Gregarina, which parasitize beetles, cockroaches and grasshoppers.

Intermediate forms of associations, we find beautifully represented in certain protozoans and termites where the symbiotic relationship apparently has reached the acme of perfection. We are less certain as to the nature of the symbiotic relationship found in the malarial parasite and the mosquito. Whether the presence of sporozoan parasite in the digestive tract of the mosquito, with the consequent penetration of the stomach wall by the ookinete and cyst formation, causes any inconvenience or injury to the mosquito remains an unsolved problem. Histological studies indicate disturbances in the tissues but nothing is known as to the ultimate effect on the duration of life of the mosquito. There is an urgent need for careful studies on this subject.

Protozoan organisms involved in parasitism and symbiosis seem generally to require access to the internal tissues, including fluid tissues, while organisms in commensal relationship to insects appear less discriminating and exacting, and are frequently satisfied with merely clinging to the outside of the host. The chitinous, sometimes punctated, or roughened surface of the insect exoskeleton lends itself admirably for attachment of certain sessile forms of protozoa.

The writer's attention was called to an interesting case of commensal relationship between certain protozoans and aquatic insects while collecting beetles in a fresh-water pond in Oakland, California. White masses, identified as colonies of protozoans of the genus *Epistylis*, were found clinging to the various parts of the body of the hydrophyllid beetle, *Tropisternus californicus*. One hundred twenty-four specimens were collected, and one hundred twenty-one of this number served as vehicles for the protozoan guest. The extent of the infestation, as well as the lack of preference for a certain location on the body of the beetle, is indicated by the accompanying micro-photograph. The illustration represents an average infestation during the peak of the season, which, in this case, was about the middle of February. The periodic occurrence of the organism is evidently correlated with the cycle of protozoan life, particularly with reference to that of the genus *Epistylis*. Specimens collected thirty days later, March 15, were almost entirely free from presence of the protozoan "hitch-hiker."

The firm attachment as well as the tenacity and elasticity of the protozoan stalk is remarkable. It was only with considerable difficulty that the organisms could be removed from the beetle and placed on the slide for microscopic examination.



The degree of commensalism in the above relationship presents an interesting subject for speculation. Generally, students of ecology consider commensalism as a more or less harmless or beneficial association between two organisms. The effects of the presence of this protozoan on the beetle is difficult to determine. It would appear that it acts as a distinct handicap to the beetle as the latter seemed to experience difficulty in raising the elytra for obtaining air as it came to the surface to replenish its oxygen supply. The proportionately large clusters of protozoans on the surface of the body would offer resistance to motion through the water and lessen the efficiency of the beetle as a diver. The clusters on the legs, particularly on the hind-legs, would render

the swimming stroke more difficult and less effective. The presence of the organism, however, seemed to have no diminishing effect on the total number or the length of life of the beetle, and they seemed to thrive equally well in the aquarium as well as in their natural habitat. The protozoan, on the other hand, derives some benefit, apparently, from the association by securing free transportation to environments favorable for food and respiration. On the whole, it would seem that the beetle is the loser in the association, as it is hard to think of any benefits accruing to it. A more thorough knowledge of the conditions of this relationship is necessary before it can be assigned to its proper category in ecological thought, and emphasizes the difficulty of corraling living organisms into empirical ecological enclosures.

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THE EXCESSIVE ABUNDANCE OF CERTAIN BEES

THE Rev. G. Birkmann resided for many years at Fedor, Lee Co., Texas, and diligently collected and studied the wild bees of this locality, finding many new to science. He has now retired from the ministry, and is living at Hufsmith, Harris Co., Texas, whence he writes me concerning the excessive abundance of a species of bee, *Protobea texana* (Friese). The case is of general biological interest, because this species, a large and beautiful insect, has only been found in Texas, and few specimens exist in collections. Thus the ability to multiply enormously, at least under certain conditions, has not caused it to be generally abundant or wide-spread. Mr. Birkmann's account, from which the following is abstracted, is partly from his letters and partly from an article contributed by him to a local paper, the *Giddings News*, of September 2, 1932.

During the last days of August Mr. Birkmann's son reported the abundance of bees in the air, making "a great ado and hubbub flying in all directions, and not only over a small area, but for quite a distance the noise is heard and the ground is full of newly dug holes." Mr. Birkmann went to the place, and confirmed the account. The bees were flying, "some of them only a half foot from the ground, others higher, so as to get over the weeds, still others pretty high up, all of them flying fast and like so many little furies, making all the noise possible to them. You could hear them at some distance, the sound was similar to that heard in the telegraph wires at times." The bees flew with such

energy and speed that they would not try to avoid any one standing in the way, but struck Mr. Birkmann's body and hat. Some of the nest holes went down several feet, but it was not always easy to measure them with a switch as they were often curved or irregular. The diameter of the holes was about half an inch. The soil dug out of the holes was of a brick red color, but the surface of the ground was sandy and deep greyish, with very little vegetation, except some plants of *Croton*, which were visited by the bees.

In all his experience, Mr. Birkmann had never known anything like it. He stepped off the ground, and found the area to be about 700 feet in length and 230 in width, with 39 holes in 100 square feet, and 46 holes in another like area. Thus "there were about 80,000 holes, and counting one pair of bees for each hole, there would have been one hundred thousand individual bees. All of the same kind." This lasted for about four or five days, according to Mr. Birkmann's observation; after that "they are in their burrows, and the females will come out to gather food for their offspring." Mr. Birkmann's grandson Roger observed that a week or ten days later "the wild flying and buzzing was renewed several times in fine sunny weather. But when there were indications of rain coming, or in cloudy weather, the bees were not out."

A similar observation was made by my wife (Wilmatte P. Cockerell) in the vicinity of Calvinia, in a dry region of South West Africa. It concerns a totally different but even more peculiar bee, the *Fidelia villosa* Brauns. She has written the following account:

The "white bees" of the desert were full of interest to us all; their adaptation to the grey world of the desert, and their apparent rarity, made us all anxious to secure specimens. Jack Ogilvie took one on the wing, and I was determined to find one at work. I had been so often successful in finding the special bees of the cactus that I felt I must have the same success with these bees of the African desert. My husband thought that probably I should find the bees visiting the small white-flowered *Mesembryanthemum*, then blossoming in great numbers on the desert about us. But we always find collecting poor when there is a great profusion of blossoms; it almost seems that the bee population finds it impossible to keep up with the flowers at the height of their blossoming season. Also we found that the *Mesembryanthemums* do not have special bees as do most of the cacti, so while I collected numerous bees on the white flowers, I did not find a single *Fidelia*, though I saw two or three flying with a curious drifting motion over the sands or the flowers. One day Alice Mackie and I were collecting; it was a very warm day I remember and we had engaged to have a car meet us

at a certain cross roads to save the walk through the grilling heat. We hurried across the sands, when suddenly we were arrested by a great buzzing sound. There must be a swarm of hive bees about, was my first thought but it proved to be the rare *Fidelia* in numbers, nesting in the sand. It was a wonderful sight, and we stayed to enjoy it for a few minutes, promising ourselves a real study of the colony the next day; but that night there was a heavy rain, and we were obliged to leave before another day of sunshine would get the *Fidelia* to work again. But during the time we were at the nest place we noted that the area was almost as great as a city lot, say 50 feet by 100, and quite full of nest tunnels, arranged of course in groups, since some of the ground was better adapted for nests than other parts. The bees were flying about over this area with the greatest activity, with the curious drifting flight that I had noticed on the few individuals I had seen before. Some of the tunnels were being provisioned, and we saw the females entering with their loads of pollen. Other tunnels were being made and the sand was flying as the little miners carried on their excavations. Here and there I thought I caught sight of a grey fly hovering about. We took a few specimens, and hurried on to our waiting car; had we known that we should have no chance to visit the colony again, the car might have waited. I shall always regret not making a thorough study of this unique bee town.

One might suppose that insects showing so much vigor and such powers of reproduction would spread far and wide, competing successfully against the more ordinary types of bees. This is not at all the case, and it is no doubt true that these insects flourish only in a special kind of environment. Both *Fidelia* and *Protoxaea* appear to be old types, now limited to relatively small areas. The Oxaeidae consist of two genera, *Oxaea* Klug, 1807, and *Protoxaea* Cockerell and Porter, 1899. Owing to the well developed maxillary palpi, we must conclude that *Protoxaea* is the older type. *Oxaea* has ten known species, ranging from Argentina to Mexico. *Protoxaea* consists of *P. gloriosa* (Fox), New Mexico and Arizona; *P. texana* (Friese), first collected by Boll at Dallas, Texas; *P. vagans* (Fox) from Lower California; and *P. impunctata* Cockerell, from the Federal District, Mexico.

The Fideliidae are entirely South African, and consist of *Parafidelia* Brauns, with *P. friesei* Brauns, Bechuanaland, and *P. ornata* Cockerell, Damaraland; and *Fidelia* Friese, with eight species, *F. paradoxa* Friese, *F. villosa* Brauns, *F. kobrowi* Brauns, *F. braunsiana* Friese, *F. major* Friese, *F. aliceae* Cockerell, *F. ogilviae* Cockerell, and *F. alba* Cockerell. They occupy the driest regions of S. Africa, but one has been found in the Transvaal.

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THE AMERICAN SOCIETY OF ZOOLOGISTS EMBRYONIC DETERMINATION

MOSAIC *VS.* EQUIPOTENTIAL DEVELOPMENT¹

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It is interesting to note how old questions and controversies crop up again and again in slightly new form in the progress of science. One of these ageless questions concerns the essential nature of development. In the eighteenth century it was "evolutio" or preformation *vs.* epigenesis. In the late nineteenth and early twentieth centuries it was mosaic *vs.* equipotential development. To-day it is self-differentiation *vs.* embryonic induction.

We now know that these two are not antagonistic or mutually exclusive, but that each is true in part, and the problems of embryonic determination are chiefly those of the time, place and manner in which each is operative. After the absurdities of extreme preformation came the absurdities of extreme epigenesis. Up to the last decade of the nineteenth century orthodox biology held to a rigid belief in extreme epigenesis, and if it did not generally go as far as did Alex. Goette (1875) in claiming that the egg of the toad is unorganized and non-living material until after fertilization, it held firmly to the faith that embryonic differentiation first appeared in the late cleavage or blastula stages and was entirely correlative. The dictum of Driesch was generally accepted by embryologists, namely, that "the fate of a part is a

¹ Paper read at the symposium on "Embryonic Determination" before the American Society of Zoologists, Atlantic City, December 30, 1932.

function of its position." It is true that there were always representatives of the opposing view of self-differentiation, but in the main they were as voices crying in the wilderness. It was generally held that morphogenesis began with the germ layers of the gastrula. Driesch claimed that the cleavage of the egg was a mere "sundering of homogeneous materials capable of any fate," and W. K. Brooks, at the time (1891) when I presented my doctoral thesis to him, held that "cleavage is a mere vegetative duplication of parts without morphological significance." On the other hand, Wilhelm His (1874) argued on purely logical grounds that there are "Organbildende Keimbezierke" in the blastoderm of the hen's egg and W. Roux (1884), as a result of his notable experiments on the frog's egg, held that from the four-cell stage on the development was a "mosaic work of four independently developing pieces."

Largely under the influence of Professor Whitman, the work of the Woods Hole school of embryologists proved that embryonic differentiation appears in the early cleavage stages and that the early blastomeres of many different species are not totipotent; that even in some unsegmented eggs there are regions and substances that are already differentiated and destined under typical conditions to give rise to specific parts or organs. But on the other hand there were certain groups of animals, particularly coelenterates, echinoderms, nemertines, amphioxus, teleosts and amphibians in which no such early differentiations were shown, and consequently I proposed in 1897 that the former type should be known as "determinate," the latter as "indeterminate." Indeed, I once suggested rather facetiously that "every egg is a law unto itself," and this fly was embalmed in the amber of E. B. Wilson's book on "The Cell." For a time there seemed to be a lack of any general principles as to the time and manner in which embryonic differentiations appear, and one eminent physiologist informed me that

the study of cell-lineage had been a blind alley which led nowhere.

More recent work has shown that many of these so-called indeterminate types of cleavage are not really such, or at least that the cleavage cells are not composed of undifferentiated cytoplasm. For example, in one of the most indeterminate types, the scyphomedusan *Linerges*, I found (1906) in the unsegmented egg a nearly concentric arrangement of three cytoplasmic substances, namely (1) a peripheral hyaline layer, thickest at the animal pole, which later forms the outer ciliated portion of the ectoderm cells, (2) within this a yolk-rich layer, thickest at the vegetative pole, which later forms the inner ends of the ectoderm cells and most of the endoderm cells, and (3) a central gelatinous area, which later becomes the blastocoel jelly and which by absorbing water causes the enlargement of the blastocoel. It is evident that with such a concentric arrangement of egg substances, large fragments from any portion of the egg would contain portions of all these substances.

The echinoderm egg, upon which more experimental work has been done than upon any other, was held until recently to be a striking example of indeterminate cleavage. Driesch (1891, 1893) at one time maintained that cleavage cells were all alike, "like balls in a pile," but the later works of Runnström (1914), Hörstadius (1928) and Plough (1927) show that as early as the 4- and 8-cell stages the blastomeres of various echinids show marked differentiations. On the other hand, Tennent, Taylor and Whitaker (1929) have proved that before fertilization the egg of *Lytechinus* may be cut in two in any plane and the fragments remain capable of fertilization and complete development. Still more remarkable is the fact that at the time of operation the polar differentiation of this egg is not fixed, but in each half the chief axis is established perpendicular to the plane of section. Evidently in the interval between the stage at which they

operated and that with which Hörstadius worked a notable degree of differentiation has arisen.

The cytoplasmic substances of many ovarian eggs have a more or less concentric arrangement, which undergoes polar concentration and differentiation at the time of maturation or fertilization. Thus in the eggs of many gasteropods little if any cytoplasmic differentiation is visible until the germinal vesicle dissolves and its clear, hyaline contents spread out over the animal pole and extend about two thirds of the way to the vegetative pole; this hyaline substance goes in large part into the three quartettes of ectomeres, while the yolk-rich substance of the vegetative hemisphere gives rise to the endoderm (Conklin, 1910). The primary mesoderm comes from the left posterior macromere, which bears the polar lobe, and this lobe is visible at each division from the first maturation until the second cleavage; if it is removed mesoderm fails to develop (Crampton, 1896). Therefore, before the first cleavage, substances destined to the formation of all three germinal layers are already present and localized in the gasteropod egg.

In the ascidian egg, which is one of the most highly differentiated types known, the different cytoplasmic substances before maturation are nearly concentric in position; there is a granular peripheral layer which later goes into the mesoderm and may, therefore, be called "mesoplasm," a hyaline layer thickest at the animal pole, especially after the escape of the contents of the germinal vesicle, which may be called "ectoplasm," since it gives rise chiefly to ectoderm, and an inner yolk-rich layer the "endoplasm," which largely goes into the endoderm. After fertilization and before the first cleavage these concentric layers rapidly take the characteristic positions which they occupy in the embryo and larva. The polar differentiation is accentuated by the flow of mesoplasm to the vegetative pole, then the mesoplasm forms a crescent around the posterior side of the egg, parallel with the first cleavage spindle, and the chorda-neuroplasm

forms a crescent around the anterior side; the endoplasm lies on the vegetative side of these crescents and the ectoplasm on the animal-pole side. Thus bilaterality and the definitive pattern of localization are established.

Destruction of individual cleavage cells of the ascidian egg, while others survive, demonstrates that these cells are specifically differentiated as early as the 2-cell stage, —a right or left $1/2$ blastomere develops only a right or left half-larva, anterior or posterior $2/4$ blastomeres develop into anterior or posterior half-larvae, etc. Relatively weak centrifuging of ascidian eggs proves that yolk, pigment and mitochondria may be displaced without preventing normal development, but if the localized areas of hyaloplasmic substances are displaced by strong centrifugal force, corresponding organs of the larva are displaced. In a recent masterly work Daleq (1932) has shown by numerous experiments, in which eggs were cut in two in various planes, that formative substances of the egg of *Ascidiella* are definitely localized before fertilization in essentially the same patterns as in *Styela* and *Ciona* after fertilization.

Cerfontaine (1906) held that the *Amphioxus* egg was bilaterally symmetrical before fertilization, and I have found that the fertilized but unsegmented egg of *Amphioxus* has a pattern of localization essentially like that of the ascidian egg, namely, a mesodermal crescent around the posterior side, a chorda-neural crescent around the anterior side, an ectodermal area ventral to these crescents and an endodermal area dorsal to them. As in ascidian eggs, the first cleavage is always in the plane of bilateral symmetry and divides these crescents and areas into exactly equivalent halves, but the second cleavage, which is at right angles to the first, separates most of the chorda-neural crescent in front from the mesodermal crescent behind. Experiments prove that only the first two blastomeres are equipotential; lateral $1/2$ or $2/4$ blastomeres regulate by the peripheral substances of the two crescents flowing in over the originally median side

so that these isolated blastomeres become bilaterally symmetrical. The further development of such isolated blastomeres is typical, except for size. Isolated anterior or posterior blastomeres of the 4-cell stage, or any $1/4$ blastomere, do not restore the formative substances that are lacking at the time of isolation and the further development of such blastomeres is more or less partial. Anterior blastomeres may form chorda and neural plate as well as a small amount of mesoderm and an atypical gut. Posterior blastomeres form an atypical gut and mesodermal somites, but no chorda or neural plate. Complete regulation occurs only in the restoration of bilateral symmetry in right or left $1/2$ or $2/4$ blastomeres. Except for this regulation, development of isolated blastomeres in *Amphioxus*, as in ascidians, is a mosaic work.

The development of partially separated and rotated blastomeres of the 2- and 4-cell stages of *Amphioxus* demonstrates that all the poles and axes of these blastomeres are irreversibly fixed as early as the first cleavage. All possible dislocations and rotations of such blastomeres take place in my experiments and the twin larvae that develop from these are united in corresponding ways. Usually anterior blastomeres of the 4-cell stage separate more readily than posterior ones and in the later development such blastomeres give rise to larvae double anteriorly and single posteriorly. But the antero-posterior axes of the two halves may vary from a condition in which they are nearly parallel to one in which they are at right angles to each other or even to a complete reversal of the chief axes in the two halves. Likewise the dorso-ventral axes in the two halves may run in the same direction or they may be completely reversed. All of these dislocations of embryonic axes are caused by the partial separation and rotation of blastomeres in the 2- or 4-cell stage.

Such dislocations and rotations of blastomeres of the 2- or 4-cell stage sometimes bring the ventral side of one

blastomere into contact with the dorsal side of the other, or the anterior side of one into contact with the posterior side of the other, but there is no indication that the neural plate and notochord of one act as an "organizer" in modifying the development of the other blastomere. In short, there is here no embryonic induction, such as Spemann and his associates have demonstrated in the case of amphibian embryos,—perhaps because each half of the *Amphioxus* egg contains its own organizer and the two halves develop synchronously. On the other hand, there is here the most convincing evidence of the early embryonic determination and self-differentiation of the blastomeres of *Amphioxus*.

In conclusion, the newer experimental work on embryonic determination indicates that in many cases where it was formerly thought that blastomeres were equipotential they are not really such. Localizations of different formative materials take place as early as the 8-cell stage in practically all animals, and in many forms such localizations take place as early as the first cleavage or even earlier. The earliest form of localization of different substances is more or less concentric, later a polar localization is clearly marked, and still later bilateral, antero-posterior and dorso-ventral localizations are evident. In a number of animal phyla all these localizations occur before the first cleavage, in others they appear only during the course of cleavage.

What is the formative agent in embryonic differentiation? Without attempting to find the *primum movens* we may conclude that if there are material differences in areas and cells it is not necessary to resort at once to some immaterial agent to account for their differentiation. It is impossible to understand, *i.e.*, to make intelligible, development except as a result of the formation and localization of different material substances. Indeed development consists in morphological division of substances and physiological division of labor. Cytologists and geneticists have made notable advances in the

study of the distributions of differentiated chromosomes and genes in the germ-cells, but the cytoplasm of the egg cell has frequently been regarded as mere foodstuff for these nuclear elements, in spite of the fact that practically all differentiation takes place in the cytoplasm. We are beginning to realize that the central problem of development lies in this relation between the genes and the cytoplasm, and that the cytoplasm is something more than mere nutritive "stuff."

Throughout my scientific life I have been waging a fight for the recognition of the importance of the cytoplasm of the egg. I was once introduced to a scientific audience as "the friend of the egg" when it had few friends, and I am now happy to find that its friends are increasing both in numbers and in importance. Fashions change in biology as well as in dress and they often move in cycles. If one only goes slow enough the rapidly moving procession may come up from behind and once more "the last may be first."

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ON THE ORIGIN AND DIFFERENTIATION OF THE SEXUAL GLAND¹

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THIS discussion is to deal with the physiology of development of the sexual gland with particular reference to the processes of determination. The special topics to be considered are (a) the nature of the organization of the gonad-forming area at various stages of its early development, (b) the relationship of the primordial germ cells to the origination and differentiation of the gonad, and (c) the mechanism controlling the differentiation of the germ cell.

I

The evidence that the organization of the gonad-forming area of the chick varies from stage to stage has come from a study of the differentiation of the gonad-forming area in grafts to the chorio-allantoic membrane. In 1927 it was discovered in such grafts that the gonad rudiment of the genital ridge stage, although morphologically indifferent as to sex, is capable of undergoing self-differentiation into a gonad of specific sex. Invariably the right genital ridge differentiates into a testis or right ovary, and the left differentiates into a testis or left ovary. The rudiment is thus found to be specifically organized as to sex and in the case of the female as to laterality also (this conclusion as to lateral differences in organization is made possible by the well-known and striking differences in the structure of the right and left ovaries in birds). The genital ridge is, then, not physiologically but only morphologically indifferent as to sex.

These findings led quite naturally to an analysis of the potentialities of the gonad-forming areas of stages earlier than the genital ridge. These include (a) stages prior to

¹ Paper read at the symposium on "Embryonic Determination" before the American Society of Zoologists, Atlantic City, December 30, 1932.

the formation of the germinal epithelium, when the gonad material is prospective (donors having from 29 to 34 somites) and (b) stages at which a germinal epithelium is visibly differentiated, the earliest beginning of the gonad rudiment (donors having from 35 to 41 somites). In all these stages germ cells are present in the gonad-forming areas.

With respect to the differentiation of gonad, the results of this analysis are summarized briefly as follows:

(1) The gonad-forming area, just prior to as well as during the formation of the germinal epithelium, has the power to form a gonad of specific sex but with distinctly less frequency than the genital ridge (39 per cent. as against approximately 100 per cent. for the latter).

Furthermore, a gonad of specific sex differentiates from the gonad-forming area of a stage prior to the origin of the germinal epithelium in 20 per cent. of the grafts, whereas after its formation in 57 per cent. of the grafts.

It is thus quite evident that a gradual increase in the frequency of differentiation of a gonad of specific sex occurs as the morphogenesis of the gonad rudiment takes place.

(2) A gonad-like body of undetermined sex forms commonly (56 per cent.) in grafts of the gonad-forming area, whereas it rarely arises in grafts of the genital ridge. Prior to the 31-somite stage this is the only type of gonad thus far formed in eight grafts examined. Several grades of organization of these bodies are recognized, ranging from a mass of stroma containing few germ cells to a definitely circumscribed body consisting of stroma, sex cords of germinal and non-germinal cells.

(3) The gonad-forming area yields somewhat frequently multiple gonads, whereas a well-defined genital ridge gives a single gonad, as a rule.

(4) The size of the gonad of specific sex which arises from the gonad-forming area is smaller than one from the genital ridge.

It is thus seen that a progressive change in the developmental potentialities of the gonad-forming area occurs as it transforms into a genital ridge. The question now arises as to what this signifies. The gradual increase in developmental capacity is interpreted as indicating a progressive change of some sort in the organization of the gonad-forming area. That is, it is an index of an ascending organization, measuring apparently real differences in the specialization of the gonad-forming area at various stages in development. The gonad-forming area may have at first little or no specific organization as to sex. It merely possesses at the time of isolation an organization, conditioned by its position in the whole, which under favorable conditions in the graft may acquire the specific potentialities of sex by means of a series of processes. If, on the other hand, conditions (developmental mechanics, etc.) are so unfavorable as to interfere with morphogenetic processes, the harmonious whole of the implant and so forth, such a chain of processes may be halted at different stages, resulting in various grades of gonad-like bodies undetermined as to sex. In other words, the process of epigenetic development continues to a variable degree depending upon the developmental harmony within the implant.

Prior to the 31-somite stage the gonad-forming area may possess an organization of a lower grade still, since it had thus far failed in eight grafts to differentiate into a specific sexual gland, giving rise only to a gonad-like body. Similarly, Corinaldesi (1927), using a different method of analysis, was unable to obtain a gonad from the prospective gonad area of earlier stages.

The conclusion is therefore reached that the gonad-forming area during its initial development exhibits an ascending organization which finally attains at the genital ridge stage a fully determined condition.

II

The nature of the problem of the relationship of the primordial germ cells to the origin and differentiation of

the gonad may be pictured by two questions. Are primordial germ cells necessary for the origination of the gonad-forming area? Do germ cells have a stimulative (inductive) action on non-germinal cells and tissues, *viz.*, upon mesenchyme or coelomic epithelium?

Masses of germ cells may occur in abundance in the mesenchyme (or spaces within it) of grafts of the gonad-forming area, yet they fail to form a gonad or to cause with few exceptions a condensation of the surrounding mesenchyme. In such positions germ cells merely undergo repeated multiplication, exhibiting no "organizing power." A similar result, according to Dantschakoff (1932), is seen in grafts of the "germ-cell crescent" of Swift, grown in the extra-embryonic coelome of an early blastoderm or in the chorio-allantoic membrane, with the exception that in the latter a strong thickening of the mesenchyme is brought about. The apparent reaction of the mesenchyme to the germ cells is probably not specific, since a similar thickening is known to occur in response to non-germinal cells and tissues.

That germ cells may have an activating effect upon coelomic epithelium, however, is indicated. In a number of grafts of the gonad-forming area germ cells occur in abundance beneath the coelomic epithelium overlying the Wolffian duct or ostium of the Müllerian duct. This epithelium, thin and non-gonad forming in the normal embryo, thickens into a layer (possessing a few germ cells) which simulates strikingly a germinal epithelium. The underlying mesenchyme may even condense into a stroma, yet the essential components of an avian gonad, namely, the sexual cords, fail completely to invaginate.

On this problem the recent experiments reported by Dantschakoff (1931) have a bearing. According to her, following the destruction by electric cautery of the prospective gonad region lying just behind the omphalo-mesenteric arteries of the two-day chick embryo, the primordial germ cells ("die entodermalen Wanderzellen") settle down in more anterior levels of the splanchnic

mesoderm. Furthermore, when such "strange" splanchnic mesoderm, after it has reached an age of four or five days, is transplanted to the chorio-allantoic membrane it may give rise to a typical testis possessing germ cells. Since in all probability the gonad-forming areas normally extend anterior to the burned region, these experiments can not be regarded as demonstrating a stimulative action upon coelomic epithelium at a strange level.

On the contrary, the origin and differentiation of the germinal epithelium appear to be independent of primordial germ cells. This interpretation was first suggested by the formation of a testis with sterile sexual cords in grafts of the gonad-forming areas from 33- and 37-somite donor embryos. Since germ cells occur in the same grafts and consequently could have furnished the initial stimulus to the coelomic epithelium, this result can not be considered as decisive. The proof, however, of the independent origin and differentiation of the gonad—at least of the testis—has come from a study of grafts of whole blastoderms of early somite stages following the removal of the germ-cell crescent of Swift. In a number of such grafts a small testis has developed. In all these cases the sexual cords are typical in every respect, except that germ cells are entirely lacking.

In *Amblystoma* the reverse situation holds. Humphrey (1928) has presented evidence from graft studies that the germ cells must be aggregated in sufficient numbers next to or projecting into a coelomic cavity before a gonad forms. According to him the primordial germ cells play the dominant rôle in the early development of the gonad.

III

Whether or not a germ cell differentiates in the male or female direction depends apparently upon its tissue environment. In chorio-allantoic grafts of the gonad-forming areas two types of germ cells, differentiated and undifferentiated, are distinguishable. The former are

found in mesenchyme and in the medulla of the right and left ovaries; the latter occur in the sexual cords of the testis, cortical cords of the left ovary, and in certain gonad-like bodies. The undifferentiated cell is characterized by the presence of numerous dark-staining granules (presumably mitochondrial in nature) *uniformly* distributed in the cytoplasm. In the differentiated cell, on the other hand, the granules are *aggregated* into a group situated at one side of the nucleus. This difference in granular distribution in the cytoplasm of the germ cells appears to be an indicator of differentiation. Slight differences in the cellular differentiation of germ cells are seen in the two sexes. In the male sexual cord the granules of the germ cells are finer and lighter staining than they are in the female sex-cells of the cortex. Other differences are also noted. In the testicular cord the germ cells are large and occur singly, while in the cortex they are distinctly smaller and grouped.

The fact that the germ cells in the mesenchyme remain unchanged—*i.e.*, like the original germ cells, while those which come to be situated within sexual cords, either male or female, undergo structural changes—furnishes convincing evidence that they are dependent upon a specific tissue environment for their differentiation. The germ cell, although specialized in a general way as a sexual cell and thus differing from non-germinal cells, is really at first sexually indifferent or neutral and remains in this condition in such non-sexual tissues as mesenchyme. It undergoes specific cellular transformations as to sex only in the specific tissue environment of the sexual cord. In a testicular cord the transformation goes in the male direction, whereas in a cortical cord it goes in the female direction.

The apparent capacity of the indifferent germ cell to differentiate in either the male or female direction in the graft indicates that it possesses bisexual potentialities. This has been beautifully demonstrated in the female

fowl by an experimental study of the right ovary (see Domm, 1929). It has been found that if the left ovary is removed during the period when germ cells are still present in the right ovary, *viz.*, from the time of hatching until about the third week, it transforms into a testis containing male sex-cells; if removed after they have disappeared a sterile testis differentiates. It would appear from these experiments that (a) the germ cells remain indifferent in the medullary cords of the normal right ovary, and (b) when the inhibiting influence of the cortex of the left ovary is removed, the latent male potencies of the medullary cords become expressed in the form of seminiferous tubules, the germ cells therein undergoing a specific differentiation into male sex-cells and even into their definitive form, the spermatozoa.

Similarly in the amphibians Witschi (1929) has brought forward evidence of considerable weight that the germ cell is dependent upon a specific tissue for its differentiation into male and female sex-cells. According to his "theory of localized sex-differentiators" the sex-differentiating factor is localized in the cortex and medulla. The cortex is a female differentiating system, whereas the medulla is a male differentiating system. To put it in other words, the differentiation of the germ cell is determined by its location within the gonad.

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SOME DIFFICULTIES OF THE DETERMINATION PROBLEM¹

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THE general title of this symposium leads me to comment on the origin and significance of the concept "determination" and to point out some of the difficulties encountered in its use. This word has come very much to the fore in the past few years, born, as it were, under an unlucky star and reaching maturity at a time when physics has nerved itself to cast aside the notion of causality in a strict sense and to talk indeterminism—to the comfort, I may add, of those wistful thinkers who see in this modern development of science a ground for their belief in the freedom of the will. It is as if a sort of Presbyterian biology were coming upon the scene just as physics is about to go over to the Baptists.

The preformationist embryology meant not merely that the qualities of the egg "determined" those of the completed organism, but even went so far as to assert that the organism was actually rolled up in the egg. So far as I am aware, however, the word "determination" was not generally applied in this sense. It, or its German equivalent "*Bestimmung*," came first into use in embryology in relation to the supposed action of internal and external factors in fixing certain qualities of the embryo, as, for instance, in Roux's (1883-87) studies "*Über die Bestimmung der Hauptrichtungen des Froschembryo im Ei.*" Contrary to the general impression, Roux used the expression with reference to epigenetic as well as to preformational processes.

In fact, the theory of epigenesis, like the theory of preformation, postulates that the qualities of the egg lead to or determine the qualities of the organism that de-

¹ Paper read at the symposium on "Embryonic Determination" before the American Society of Zoologists, Atlantic City, December 30, 1932.

velops out of it, and that all the possibilities of development, presupposing of course a suitable environment, must be implied in some way in the egg. Inasmuch as this theory assumes that the localized qualities of the mature organism do not exist as such in the germ but arise gradually through the interaction of parts with one another and with the environment, that is through "Auslösung," or release of potencies, these factors are conceived as fixing or determining the qualities of other parts which are brought under their influence.

Though the expression was earlier used sporadically,² it was Korschelt and Heider (1902) who formally gave the name "determination" to this field of study. At the beginning of the chapter entitled "The Problem of Determination" they define it as follows (p. 81): "We count under this rubric the whole cycle of questions that deal with the disposition of the constituent parts of the embryo with reference to their future fate. Accordingly, it has to do with the origin, nature and localization of organ-forming factors—a field that embraces the fundamental questions of embryology and although already taken up from various sides, one that still remains very much in the dark." This was written in 1902. In 1932 I fear the subject still remains in the same condition.

The data of genetics and cytology show that the characters of the organism are represented in some orderly arrangement in the chromosomes. The results of experimental embryology indicate, though less conclusively, that the whole of the egg cytoplasm carries some features

² For example, by Wilson (1893, "Amphioxus and the Mosaic Theory," p. 617) and by Driesch (1894, "Analytische Theorie," p. 76). Conklin (1897) in his paper, "The Embryology of *Crepidula*," first proposed the phrase "determinate cleavage" to designate such types of cleavage as are found in mollusks, annelids, tunicates, etc., in which the pattern is definite and leads to the view that the history of each cell is predetermined (p. 190). The term "determinant," introduced by Weismann (1892), was used to denote a hypothetical unit of the germ plasma, resembling to some extent the "gene" of modern genetics. These determinants were supposed to fix or establish, in a purely preformationist sense, however, the qualities which later appear in the course of development of the organism.

that are fundamentally similar throughout and that the several regions differ from one another mainly in the relative intensity of their various activities. Accepting these data, the general problem of development is how such a system is transformed into one in which the various parts differ more fundamentally according to region, as they do in the mature organism. The problem is thus in first instance one of segregation, as has recently been emphasized by F. R. Lillie (1929). In other words, one of the early important steps of development is the separation locally of qualities that are at first combined or mixed throughout the whole egg cell. It is what Driesch called the distribution of potencies (*die Verteilung der Potenzen*).

According to common usage, "determination" is mainly applied to processes that occur after a certain amount of segregation has taken place. Since it is in reality an invisible differentiation, it is essentially what Roux (1885) had in mind when he expressed the opinion that the key to the causal interpretation of embryonic development lies in the answer to the question whether differentiation takes place by itself (self-differentiation) or whether it is the outcome of an interchange of effects between each part and its surroundings (dependent differentiation).

This is a legitimate and satisfactory, if only preliminary, statement of problem which has led to many fruitful experiments. Now it might all be well enough if we confined our use of "determination" to the processes themselves, and described the changes that take place as differentiation proceeds, but trouble begins when, as is more frequently done, we use the word to denote a state and ask the question whether an organ rudiment is "determined" or not, meaning thereby whether it is so fixed as to its capacities that it can do but the one thing that it does do. This difficulty rests upon the fact that there is no certain criterion by which this can be answered. A number of tests involving different conditions may be applied, but they frequently do not give the same answer

to the question, nor do they tell us unequivocally whether the factor that is supposed to determine has ceased action or not.

The tests of determination may be classified roughly as negative and positive. Of the former, simple extirpation of the material from which an organ develops is the most readily applied. If, after removal of the rudiment, *e.g.*, that of the balancer or the lens in the amphibian embryo, the organ fails to develop, the rudiment is considered to be already determined. If the organ does develop after extirpation of the rudiment, the process is frequently spoken of as regeneration or postgeneration, but this does not preclude a considerable degree of "determination" or specialization in the primordium itself, for the same may in such cases often give rise to the proper organ when grafted to another place. The case is somewhat more complicated when, after extirpation, another piece of tissue is put into its place. Failure of the organ to develop does not then necessarily signify more than that the particular substitute lacks the power to form the organ, but the actual development of the organ means that the influences of surrounding tissues are still active. Such phenomena have led to the concept of "double assurance." Further knowledge of what is happening under such circumstances may be obtained, either by simple extirpation of the surroundings, more particularly the substrate, or by substituting for the normal surroundings something of a different nature.

The most usual positive test of determination is the performance of a given primordium when removed from its normal environment and placed in new relations in the embryo. In applying this test, however, differences are frequently shown between regions that are active in influencing what is near them and other regions that are more neutral. Ectoderm, taken from over the mandibular arch of the neurula or early tail bud stage in *Amblystoma punctatum*, gives rise to a balancer when transplanted to various parts of the head but will not do

so when grafted upon the trunk (Harrison, 1925). When taken in later stages, however, it gives rise to a balancer here also. Some continued action of the substrate seems necessary before the power to form a balancer becomes fixed in the ectoderm. An ear placode, taken at about the time of closure of the neural fold, develops into a highly differentiated and sometimes almost perfect ear when grafted between ear and eye, but it becomes only a small rounded vesicle without sensory areas when grafted to the limb region (Yntema, 1933). This difference can be due, if at all, only in part to mechanical factors, since ear placodes grafted at slightly later stages develop into much larger vesicles with well-defined sensory areas. ••

A more rigorous method of testing whether an organ rudiment is "determined" is by means of its powers of self-differentiation when planted out on a substratum, such as the chorio-allantoic membrane, that affords proper nourishment without exerting any specific morphogenetic stimulus. The most radical method is complete separation of the part to be tested from any other living material, as in the separation of blastomeres or in the explantation of the part under tissue culture conditions, but these methods also yield different answers to the same question.

A piece of medullary cord, taken prior to visible histological differentiation and isolated in a saline solution or in serum without contact with a solid substance, remains relatively inert, while a similar piece, placed in plasma, which later clots, differentiates into nerve fibers which extend far out into the medium (Harrison, 1914). The only difference here is a purely mechanical one, the chemical constitution of the medium being the same in the two cases. It is the presence or absence of a solid framework that decides whether the nerve fibers grow out or not.

The varying results in the above cases are due to different factors that are secondary to the processes of de-

termination. The effectiveness of such factors is brought out in an even more striking way by recent experiments made to test the self-differentiating powers of the various areas of the amphibian gastrula and early blastula.

These experiments have been carried out by Kusche (1929) and by Bautzmann (1929b) independently by implanting small pieces of the Triton embryo into the enucleated eye socket of a young larva, and by Holtfreter (1931), who explanted similar pieces to the body cavity or to a saline medium *in vitro*. The results are astonishing. First there are striking differences, depending upon the method of isolation used, especially in the ability of presumptive ectoderm to give rise to nervous tissue; which, according to Holtfreter, takes place in the body cavity but not in the saline medium. Secondly, all three investigators agree that what Bautzmann has termed "bedeutungsfremde Selbstdifferenzierung" occurs. For example, notochord and mesoderm may differentiate out of pieces that would ordinarily give rise to neural plate; nervous system may arise from presumptive abdominal ectoderm and ectoderm from presumptive neural plate. Pieces taken from the region of the organizer, if relatively large, tend to develop into complex bodies, consisting of foregut, notochord and mesoderm or of notochord, mesoderm and nervous tissue, according to the exact location from which they are taken in the early gastrula stage, but smaller pieces tend to differentiate into pure notochord, muscle or a vesicle of foregut epithelium. In general, according to Bautzmann, the region of the organizer is characterized by equality between its prospective potency and its prospective significance, although Holtfreter does find that it can give rise to nervous tissue, as well as to notochord, mesoderm and foregut epithelium. From these experiments one can not fail to conclude that the isolated part tends to form a greater variety of structures than when left in place in the embryo. Its diverse potencies may thus be realized

without the influence of an organizer acting from without.

Let us consider now certain invertebrate embryos in which determination is said to take place very early—during cleavage or even before. For instance, in the ascidian egg, as Conklin (1905, 1931) has shown, the fate of the cells in early cleavage is already determined in accordance with the segregation pattern of the cytoplasm in the unsegmented egg, which becomes fixed during maturation and fertilization. Disturbances in the arrangement of the cytoplasmic masses or of the blastomeres, produced by strong centrifuging, by alteration of the cleavage planes or by injury to single blastomeres, result in corresponding abnormalities in the mature larva. According to Daleq (1932), the future constituents of the embryo are localized, in part at least, even in the unfertilized egg, before the final arrangement of cytoplasmic substances is reached. These are facts well established by varied and convincing experiments from which one would naturally infer that the adult ascidian would have little or no power of regulation. Yet what does the study of asexual reproduction and regeneration teach?

It has long been known that certain organs, such as the ganglion and the walls of the peribranchial space, do not originate in the bud in the same manner as in the embryozoid. In the matter of regeneration Driesch (1902) showed that in *Clavelina* complete and perfect restitution takes place in either direction from a cut surface at practically any level. Even a fragment of the highly differentiated branchial basket may regenerate a whole organism, or the branchial basket itself may be reproduced by the visceral sac. In the latter case, according to Brien (1930), the pharynx and the peribranchial space grow out from the epicardial tube, itself a derivative of the pharyngeal endoderm, and the ganglion is budded off from the wall of the right peribranchial cavity. This agrees, in some measure at least, with the process of budding in

some forms,³ but in *Clavelina*, according to the most recent account of P. and E. Brien-Gavage (1927), budding takes place in the stolon in quite a different manner. Here the stolon septum is mesenchymatic and is not derived from the endodermal epicardial tube, as has been generally maintained, and the whole bud, except its epithelial covering of ectoderm, is formed out of this mesenchymatic tissue, supplemented perhaps by wandering elements from the blood. Furthermore, in *Clavelina*, under adverse conditions (in winter in northern waters and in spring and summer in the Mediterranean) the ascidiozooids undergo reduction⁴ and in the stolon buds mesenchyme cells gather in immense numbers, forming loose aggregations inside the ectodermal membrane. Out of these buds new zooids are reconstituted when external conditions become favorable. Spek (1927), who has made a thorough study of the question, emphasizes the rôle played by certain vacuolated cells (*Tropfenzellen*), but P. and E. Brien-Gavage find that the morphogenetic function of reconstituting new zooids falls to ordinary mesenchyme cells, while the vacuolated cells are nutritive. Finally, in certain cases isolated branchial baskets may undergo reduction and reconstitution, as Driesch described, during which process the highly differentiated cells are destroyed and only mesenchyme (including vacuolated) cells remain inside the ectodermal sac; out of these a new zooid is formed, as in the case of the winter bud.

While some of the processes just described are not altogether clear, particularly, whether dedifferentiation and redifferentiation or actual destruction of tissues, followed by restitution out of totipotent reserve cells, takes place, it is absolutely certain that in budding and reconstitution totipotent mesenchyme cells play the prin-

³ Of the numerous papers on the budding of ascidians the following may be cited in this connection: Hjort (1895), Caullery (1895), Ritter (1896), Lefevre (1898), Seeliger (1900-06).

⁴ See papers by Giard et Caullery (1896), Kerb (1908), Schaxel (1914), Huxley (1926), Spek (1927), Salfi (1927).

cipal rôle. The segregation pattern found in ontogeny can therefore have but temporary significance. The blastomeres may be "determined," but if so, only for a time, for endoderm later has the power to form the nervous system and the peribranchial cavity, while mesenchyme has the potency to form all tissues except possibly ectodermal epithelium.

The question raised here is an old one and has been fully considered by Weismann (1892) and others in connection with regeneration. It is not sufficiently taken into account, however, in relation to embryonic development. The facts just presented go to show that the general qualities of the organism must persist in cells that may seem to have had their properties restricted to a more specific rôle, and that other capacities than those for which a cell seems to be determined in early ontogeny may be called forth under proper conditions.

Let us now leave the passive side of the process, the "being determined," and consider some of the active factors which are described as determining. One of the earliest recognized examples of these is the optic cup in relation to the lens, as originally described by Spemann (1901) in *Rana fusca*. If the eye rudiment is removed from the medullary plate before contact with the skin is established, the lens, which is normally formed from the latter, does not develop—a typical case, as we should say, of dependent differentiation. However, in closely related forms, *R. esculenta*, for instance, this does not occur (Spemann, 1907, 1912). After precisely the same operation the lens does develop, in some cases very perfectly, and it is concluded that determination takes place here at an earlier period, in which case, however, it must be some agent other than the eye rudiment that is active.⁵

⁵ The results of von Ubisch (1925) tend to minimize the above contrast, for he finds that in *E. fusca* imperfect lenses may arise and in *E. esculenta* they are not fully differentiated. Moreover, external conditions such as temperature, salinity of medium and uncontrollable individual differences seem to affect the results.

The positive test, first applied by Lewis (1904) in *R. sylvatica* and *R. palustris* and since confirmed for many other species, brings out in a more striking way the influence of the eye upon the lens. In embryos with open neural folds or with primary optic vesicle ectoderm taken from other regions is grafted so that it will lie over the eye in place of the lens ectoderm which is removed, or else the optic vesicle is implanted under the ectoderm of other regions. As a consequence, lenses, in many cases quite perfect, develop out of the overlying strange ectoderm. In *Bombinator pachypus* and perhaps in some other species there are regional differences in the ectoderm at the period marked by the close of neurulation, for ectoderm taken then from the head develops into a lens, whereas that from the trunk does not (Spemann, 1912).

One of the most baffling results came from the following experiment. If the optic vesicle is removed from the embryo of *Amblystoma punctatum* just after closure of the neural folds, the lens, as Le Cron (1907) found, does not differentiate out of the ectoderm which remains. However, take this same ectoderm away from the optic cup and transplant it to the region of the ear or the heart, instead of taking the optic cup away from it, and it develops into a perfect lens quite independently of any eye (Harrison, 1920). It is impossible, therefore, to say that the optic vesicle determines the lens or that the latter at the period concerned is determined in any absolute sense. We are justified only in speaking of what it does when certain conditions are imposed.

The emphasis upon "determiner" and "determined" leads to a very lop-sided and often erroneous view of the process, for it is questionable whether one factor can influence another without being itself changed. This becomes obvious when we inquire into the influence of the lens rudiment upon the eye. Little is known of the early activities, but later very marked effects in this direction are shown. In the absence of the lens the vitreous body is defective, the eye does not properly expand, and the

retina may become folded (Beckwith, 1927). In later stages the mutual relations between lens and eye in respect to growth rate are clearly brought out by heteroplastic transplantation of either lens epithelium or optic vesicle alone between *Amblystoma punctatum* and *A. tigrinum* (Harrison, 1929). These two species show enormous differences in their respective growth rates and in the ultimate size to which the individual grows. When a whole eye is grafted from *tigrinum* to *punctatum* it may attain double the linear measurements of the normal eye of the host. Likewise, when the lens epithelium alone is grafted, a lens that is at first too large for the eye is formed, but later an adjustment between the two takes place. The growth rate of the eye is accelerated beyond that of the normal and the growth of the lens is correspondingly retarded, until the organ, as a whole, comes to an equilibrium at a point intermediate between the extremes of the two kinds of eye and with eye-lens proportions approximately, though usually not quite, normal; but the influence of the lens upon the eye is quite as great as that of the eye upon the lens.

The most important advance in embryology of late years has been Spemann's (1918, 1924) discovery of the organization center and organizer in the amphibian egg. This is localized in a definite region, as a cytoplasmic differentiation which may be identified with the gray crescent in the unsegmented egg and later with the dorsal and lateral sectors of the border zone between the animal and the vegetative portions of the egg. It is turned in during gastrulation to form the roof of the archenteron, and afterward differentiated into pharyngeal roof, notochord and mesoderm. Its powers are extraordinary, and almost anything brought into its neighborhood is made in some mysterious way to cooperate in forming an embryo of a varying degree of completeness. It has some limitations—its capacity for self-differentiation is restricted (p. 311) and its powers of organization wane as differentiation proceeds—but at first these powers are

of cyclonic proportions, carrying away in a puff our pre-conceived notions of preformation and germ layers. They last sufficiently long to mould the whole embryonic axis and fix the various regions of the future nervous system, and are then transferred to the medullary plate itself, which thus acquires the capacity to incite by homogenetic induction the formation of a new medullary plate when grafted under presumptive ectoderm in the blastula or early gastrula (Mangold and Spemann, 1927; Mangold, 1929). Nothing is known about the reciprocal effect of the organized material upon the organizer, but I venture to predict that when this is studied it will be found not to be negligible.

The use of the term "organizer" is likely to be attended by some confusion, for the word may be readily taken to imply more than we are really justified in attributing to the thing itself. The material upon which the organizer acts is already highly organized. The explantation experiments referred to above show that it has the capacity for diversified self-differentiation. The organizer, itself a complex system with different regional capacities, merely activates or releases certain possible qualities which the material acted upon already possesses. The orderly arrangement which results depends not only upon the topography of the organizer but also upon that of the system with which it reacts (Bautzmann, 1929 and 1932; Spemann, 1931).

Besides the main organization center there exist subsidiary organizers, the general action of which is shown by the experiment first thought out (Gedankenexperiment) by Spemann (1921), and actually executed by Schotté (Spemann and Schotté, 1932; Schotté, 1932). Indifferent abdominal ectoderm of an anuran is placed over the mouth region of a urodele embryo. To the underlying cells of this region the powers of a secondary organizer are ascribed, since a mouth and suckers, indeed almost a face, are actually formed out of the grafted tissue, but it is an anuran mouth and not that of a urodele.

As Spemann is reported to have put it, the ectoderm says to the organizer, "you tell me to make a mouth; all right, I'll do so, but I can't make your kind of a mouth; I can only make my own and I'll do that."⁶ This raises a difficult question—the significance to be attached to the abstraction mouth. Though radically different morphologically and histologically, the two mouths must have something in common, for the organizer of the one, by exerting what Spemann and Schotté term a "komplexer Situationsreiz," calls forth the other out of material that would ordinarily produce something else.

What it is desired again to emphasize is that the material which becomes "organized" merely develops certain of its own qualities in interaction with surrounding factors. Other conditions may draw out other qualities. The same kind of ectoderm that produces the mouth may, as Spemann (1918) has shown, give rise to nervous tissue if grafted into the medullary plate, where it would come under the influence of a different organizer. It is therefore said to be still undetermined in the early gastrula, but this statement must be taken with the reservation that the material is not entirely indifferent, since, if isolated, as in Holtfreter's experiments, it differentiates into nervous system and epidermis. The material thus retains not only the general characters of the species but also a variety of different special potencies, some of which it may still hold long after determination in a particular direction may seem, according to some criteria, to have taken place. There is no way of finding out with certainty whether the particular quality which a cell seems to have is finally fixed, for there always may be new conditions, not yet tested, under which other potencies might be revealed (*cf.* Schleip, 1929, p. 878). Recognition that intermediate conditions may persist for a time has led to the use of qualifying terms, such as labile, reversible or partial determination, but such phrases are in fact self-contradictory and are of little real use be-

⁶ I ask Professor Spemann's indulgence for making use of this hearsay quotation.

cause not precisely definable. It is false to regard such conditions as exceptional and as requiring special explanations. They belong in reality among the most essential characteristics of developing organisms and deserve a place in our philosophy of development commensurate with their importance.

I have elsewhere (Harrison, 1921) laid stress upon the fact that differentiation takes place gradually through a series of small steps. Some of the stages of this process have been ascertained in the case of paired organs, such as the limb and the ear, but there is probably no one who would maintain that the steps in the determination or differentiation of the lens are the same as those in the case of the limb. To classify both in the same terminology does nothing to clarify our understanding of what goes on.

Experimental embryology will be placed on a sounder basis if its questions are framed more carefully. In dealing with such a complex system as the developing embryo it is futile to inquire whether a certain organ rudiment is "determined" and whether some particular feature of its surroundings, to the exclusion of others, "determines" it. A score of different factors may be involved and their effects most intricately interwoven. In order to resolve this tangle we have to inquire into the manner in which the system under consideration reacts with other parts of the embryo at successive stages of development and under as great a variety of experimental conditions as is possible to impose. Success will be measured by the simplicity, precision and completeness of our descriptions rather than by a specious facility in ascribing causes to particular events. There is always room for fallacy, even when the logical procedure may seem unimpeachable, and no conclusion in embryology is safe if based upon but a single proof. This, to some, may all seem purely formal and of no practical consequence. It is, nevertheless, important to realize that even the language of science is still bound by tradition (*cf.* Hogen, 1931) and is by no means free from anthropomorphisms

and relics of our demonology, which are difficult to escape and which may not only lend a false sense of security to our explanations but also may suggest foolish questions that never can be answered.

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FUNCTIONAL ADAPTATION AND THE RÔLE OF GROUND SUBSTANCES IN DEVELOPMENT¹

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WHEN it was first known from the work of Culmann and Meyer that the arrangement of the lamellae in the spongy bones was such as to correspond to the trajectories of tension and pressure acting on the bone, the idea of a direct influence of mechanical function on the organization of the tissues seemed to be substantiated. Even more so, since Wolff (1892) could demonstrate that the internal structure of bones subjected to abnormal strains, as in pathological cases, changes profoundly into a pattern corresponding to the new situation and supposedly adapted to it. This is what Roux (1895) then, called "functional adaptation." Later, similar phenomena have been observed also in the cartilage and in the connective tissue systems (Benninghoff, 1931). Although these phenomena were found in the adult, Roux was ready to proclaim adaptation to the functional requirements of activity as a general principle in morphogenesis. There was, however, one essential point utterly opposed to such an attempt; that is, that the so-called functional constructions, in the embryo, already exist long before there is any corresponding functional activity. So, although there are functional structures, there is certainly nothing of the kind of a functional adaptation, in the original sense, to be found in the embryo. Rhumbler (1914), later, demonstrated that the internal architecture of the embryonic bone could be explained on the basis of assuming that internal tensions arising from the differential surface growth of the bone are the organizing factors. This system of growth tensions incidentally coincides, in its main features, with the tensions

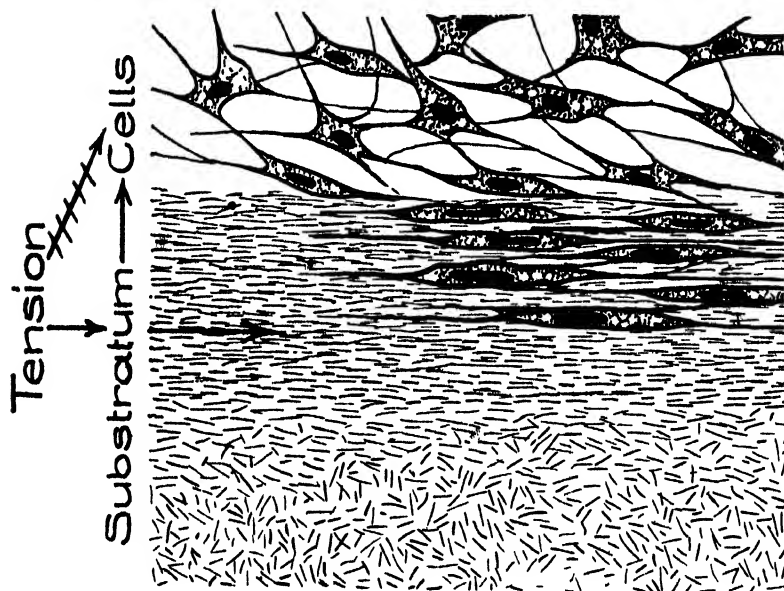
¹ Paper read at the symposium on "Embryonic Determination," before the American Society of Zoologists, Atlantic City, December 30, 1932.

that would be created by an external load, and the so-called functional structures are rather growth structures. This idea has later been corroborated by Triepel (1922), Benninghoff (1925), and others. All these authors still consider tensions to be the agents in the formation of the so-called functional structures, differing from Roux only in that they attribute these tensions to growth instead of to external load.

In a further attempt to approach, experimentally, the problem of functional structures, I started, a few years ago, a series of experiments, the results of which may be outlined as follows: The purpose was to produce functional structures in tissue grown *in vitro* outside the organism; in other words, to imitate in the tissue culture, the conditions within the organism. So, I devised a method by means of which it was possible to grow fibrocytes in a medium subjected to appropriate tension (Weiss, 1928, 1929). A thin membrane of blood plasma was coagulated in a tiny horizontal glass frame and a fibrocyte culture was put in the center. The distribution of tensions in the membrane is a definite function of the geometrical form of the frame. In the case of a triangular frame, for instance, the maximum tensions are directed towards the sides of the triangle. Under these conditions, the direction of the outgrowing cells coincides with the lines of maximum tension. In a triangular frame, we see the cells deviating from the radial direction and converging into three main bunches, directed, as are the principal tensions, toward the sides of the frame. Varied experiments of this kind always yielded essentially the same results. As in the functional structures of the organism, the cells, in their arrangement, duplicate the pattern of tension trajectories. Similar results have since been described by Huzella (1929). I further found that not only the orientation but also the intensity of cell growth is influenced in that the growth² proceeded at a higher rate in the directions of stronger tensions.

² "Growth" of a tissue culture includes both multiplication and outward migration of the cells.

Considering these experiments, one might be led to believe that they furnish decisive proof for the direct action of mechanical factors on the direction and intensity of cell growth. This direct action could be either an immediate pull or some kind of trophic stimulus in the sense of Roux. Nothing of this sort, however, holds true. It can easily be realized that there is no way in which pull could act directly on the cell. There is, on the one hand,



the coagulated medium in equilibrium with the tensional forces to which it has been subjected, and there are, on the other hand, cells freely traveling in or on it. It is obvious that these traveling cells, themselves, are as little aware of, and affected by, the tension existing in their support as a man lying on the ground is aware of, and extended by, the tension existing in the earth's crust. Unfortunately, this simple fact is sometimes entirely overlooked, so that we find in the literature effects described as tension effects in cases where tension obviously was not in play at all. Furthermore, some of our experiments bear striking evidence against the view that

tension might have acted directly on the cells. If the culture medium, having been coagulated under the influence of tension, is removed from the frame and put on a support so as to release it from the original tensions, we observe, in favorable cases, the cells nevertheless growing out in those directions which mark the former tension trajectories. Tension, therefore, can not be the immediate agent influencing the cells. We found in the experiments the cells following lines of tension which had already ceased to exist. In order to account for this result, we have to assume, first, that tension had had some orienting effect on the medium in such a way as to establish therein a system of material lines coinciding with the tension trajectories, and that these lines, then, formed pathways over which cells might migrate. The second inference is that such pathways really are preferential traffic lines for the cells, no matter whether tension persists or not.

It has been suggested (L. Loeb) and proved (Harrison, Burrows, Carrel) that tissue cells in order to be able to grow and to migrate require some solid structure as support (Harrison, 1914). In demanding a supporting substratum these cells behave like amoebae. We can not, however, enter here into a discussion of the various explanations which have been suggested to account for this peculiar behavior of cells. Terms like "thigmotaxis," although not elucidating the situation, may still be accepted for the sake of description. Probably surface tension relations on the liquid-solid interfaces along which the protoplasm extends play an important rôle in determining the thigmotactic movements. Hence, inasmuch as different kinds of protoplasm are likely to have different surface tensions, we may expect to find given interfacial structures serving as leading structures for one kind of cell while not affecting another kind at all. In any case, it is a fact that the fibrocytes in the tissue culture tend to use liquid-solid interfaces as tracks, the solid phase being represented as fibrin, the liquid phase

as serum. If, now, the particles of the solid phase are arranged in a definite orientation, the movement of the cells will necessarily be correspondingly oriented. Thus, the problem of the trajectorial arrangement of the cells reduces itself to the problem of a trajectorial arrangement of the fibrin particles. And remembering now that particles in a colloidal substratum, as has long been known, can be oriented by mechanical tension (V. Ebner, 1906), we can replace the idea of a direct action of tension on cell growth by such a conception as is represented in the following schematic picture. The ultramicrosomes or "micellae," as we may call them, using the term of Naegeli, are, in the case of organic colloids, generally of a definite polarity which in itself may be an expression of the constitution and arrangement of the high molecular compounds composing them. We may think of these micellae as being little rods of sub-microscopic dimensions (Schmidt, 1924). In a fresh colloidal sol, these little rods are irregularly distributed. Without the intervention of polarizing agents, they will aggregate to form fibrillar threads, but there will be no definite orientation in the resulting network. If, however, there is some vector force, for instance tension, acting on the system, the rods will all be oriented in such a way that their polar axes nearly coincide with the direction of that force (Ambronn and Frey, 1926). Usually, from the further agglomeration of polarized and oriented particles, oriented fibrillar structures result. As Baitsell (1915) and others were able to observe, fibrin fibers in a plasma clot subjected to tension attain even microscopic visibility. It has to be mentioned, however, that in the case of my experiments, besides a lamellar structure, no coarse fibrillation of microscopic dimensions could be detected, from which we may conclude that as a guiding structure for the cells an ultramicroscopic orientation is sufficient. We will call such an ultramicroscopic structure "ultrastructure." In our frame cultures, for example, the tensional forces acting in three main direc-

tions cause the formation of a correspondingly oriented metastructure in the clotting medium, and the outgrowing cells simply follow the preestablished pattern. However, as I stated above, not only the orientation but also the growth rate of the cells was affected. As a matter of fact, the particular ultrastructure of the medium can account for this phenomenon, too, since the growth rate depends upon the supply of liquid nutritional substances, and the distribution, circulation and supply of liquid with respect to the growing culture prove to be profoundly influenced by the ultrastructure. The displacement of liquids is facilitated along the lines of micellar orientation. Hence, growth, too, is favored along these lines.

The reason for having reported these experiments at some length is not only that they apparently furnish some clue as to the mechanism involved in the development of functional structures. Their main significance seems to lie in that they unmistakably point toward a more general principle of morphogenesis of which the so-called functional constructions are only special cases. This general principle, which may be called "principle of ultrastructural organization," as applied to the organism, could be formulated as follows: The interior architecture of the body, as expressed by the arrangement of cells and intercellular formations, is to a certain extent determined by the ultrastructural organization of the colloidal continuum which fills the interior of the organism.

Before, however, being entitled to apply to the organism this principle derived from the somewhat artificial conditions of tissue culture, one has to prove that, in the essential points concerned, the conditions in the organism and in the tissue culture are strictly comparable. The best example to prove the comparability is the process of regeneration of tendons. Tendons show the most pronounced functional structure since their cells and fibers are all oriented in the direction of pull.

From surgical experience as well as from experiments of Levy (1904), it can be learned that, after cutting a tendon, the gap between the stumps is soon bridged again by true tendinous tissue with the typical lengthwise arrangement of fibers and cells, provided a longitudinal tensional strain has been allowed to work on the regenerating tissue. In the absence of the oriented pull, the regenerated tissue does not show any oriented structure. The situation is essentially the same as in the tissue culture. There is, at first, a clot of blood formed which connects the stumps. This clot is, then, subjected to tensional stress resulting in a lengthwise orientation of the micellae, and all the immigrating cells, eventually, follow the preestablished oriented pathways. Even more striking perhaps are experiments of Nageotte (1922). He implanted in the connective tissue of adult animals pieces of tendons which had previously been fixed in alcohol, thus offering to the cells a medium with a preformed structure; and, really, cells which happened to penetrate from the surroundings into the dead graft attained, therein, such arrangement as is typical for tendons. Undoubtedly experiments of this sort bear more than a purely superficial resemblance to the tissue culture experiments. The resemblance is even closer if we take into consideration that blood plasma in the organism (Nageotte, 1931) as well as in the tissue culture (Maximow, 1929, Baitsell, 1917) is able to develop true collagenous and argyrophile fibers. Mentioning them, it must be added that they are by no means to be identified with what we consider to be the general guiding structures of cells. Where fibers are differentiated they undoubtedly can be utilized as tracks by the migrating cells. For the rest, ultramicroscopic "ultrastructures" do as well.

It is, of course, obvious that the medium in which the connective tissue normally arises in the embryo is not blood plasma, as in the case of tissue culture or wound healing; however, in all features that concern organiza-

tion it is similar to blood plasma, similar especially in that its micellae are polarized and hence capable of being oriented along the lines of force. This medium is known in later stages as the ground substance of the various organs. It appears in earlier stages as the ground substance of the mesenchyme and can even be traced back to still earlier, pre-mesenchymal, stages as mesostroma, filling the spaces between the germ layers (Snessarew, 1932). And it may be that there is some substance present even in the unsegmented egg playing an analogous rôle. At least, the way in which Lillie (1909) speaks of the "ground substance" of the egg suggests this possibility. There is still much discussion about the origin and nature of the ground substance. Some believe that there is no ground substance which is not part of living protoplasm, and some others believe that ground substances are only dead secretions of cells, like mucus. Between the two extremes, we find all intermediate shades of opinion. It is not unlikely that some time it will come to be realized that the facts are such as to preclude a uniform solution of the problem. There is one thing, however, to be considered as certain. That is, that the properties on which the formation of ultrastructures is based exist in every protoplasm. We find them at work in the processes of mitosis, in the formation of intracellular tonofibrillae, myofibrillae, neurofibrillae, etc. But it is equally certain that substances which are not protoplasmic, or no longer protoplasmic, as, for instance, some body fluids, possess in that one respect the same properties. For that reason, there is, from the viewpoint of the present problem no need of further discussing the question of whether the ground substances originate as detached ectoplasmic substances or as transformations of protoplasm or as cell secretions of some kind. We may simply record one significant point in which all the different opinions are in accord, namely, that the ground substances form a continuous system all through the embryo, from the earliest stages on. This proves

that a material which is suitable to ultrastructural organization and comparable to the medium of tissue culture is present in embryogenesis.

As to the further assumption that cell arrangement depends upon structures in the ground substance, normal embryogenesis also furnishes appropriate examples. The chorda sheath,³ the cornea and the vitreous body of the eye, for instance, at first, consist only of a cell-free colloidal layer into which cells later immigrate, proceeding along the preestablished pattern of the ground-substance. Therefore, one has to admit that, in the points under consideration, conditions in the tissue culture and in the embryo are strictly comparable.⁴

Now, however, in the embryo, what are the forces which shape the ground substances, which bring about such ultrastructural organization as has been artificially induced by mechanical factors in our *in vitro* experiments? Are the factors in the embryo mechanical as well? It has already been mentioned above that many of the organ structures considered as functional structures are merely growth structures. They are caused by internal tensions resulting from the surface growth of the organ. In these cases, tension is undoubtedly the factor which impresses an oriented structure upon the ground substance. Wherever this is the case, the particular pattern of tension trajectories is determined by the peculiar form of the growing surface. This latter plays a rôle similar to that of the frame in our experiments. If, in the simplest case, the contour of the growing organ is circular, a radial structure is to be expected. That is exactly what we find in the tympanic membrane of the ear. As the

³ V. v. Ebner, *Zeitschr. f. Zool.*, 62, 1896.—The chorda sheath shows a very marked "functional structure." Cf. Tretjakoff, *Zeitschr. f. Zellforsch. u. mikr. Anat.*, 4: 266, 1927.

⁴ Harrison was probably the first to emphasize this similarity; in 1914 (*Jour. Exp. Zool.*, 17, 521) he states: "... since it has been shown that most embryonic cells are stereotropic, and that such arrangements as they assume in the embryo may often be induced under cultural conditions by reactions to solids, there is a presumption in favor of the view that this type of reaction is a potent factor in normal development also."

tensions, exerted on this membrane in post-embryonic life by sound waves, happen to coincide with the embryonic growth tensions, the final structure of the organ looks as if it were functional by origin (Benninghoff, 1931). A more irregular contour causes a more complicated pattern of tension trajectories. For this Benninghoff (1931a) brings a very instructive example. After implanting the dead scapula of a human fetus under the skin of a rabbit, he observed the formation of a fibrous capsule around the graft. The arrangement of the connective tissue of this capsule was very similar to the fiber pattern found in normal scapular periosteum. During the normal development, the tensions determining the orientation of fibers arise from the growth of the scapula; in the experiment, they arise from the contraction of the connective tissue which coats the graft. Since, however, the distribution of tensions is determined by the form of the contour, and the contour being in both cases the same, the pattern of tension trajectories and, hence, the pattern of fibers is similar in both cases, too. We are confronted here with one of the simplest manifestations of a "Gestalt" principle, since we are dealing with a system of typical configuration in which the arrangement of every part is strictly dependent upon the whole. The external shape of the organ is not brought about by mere apposition of elementary parts in a definite arrangement, but, on the contrary, the arrangement of the parts, *i.e.*, the internal structure, is determined by the shape of the entire system. This is further evidenced in cases where an organ, after having undergone some pathological alteration during morphogenesis, shows internal structures which are entirely different from the normal, but which fully correspond to the new shape the organ as a whole has assumed. Cases of this kind have been described in chick embryos with malformed bones by Landauer (1929).

Like growth, the mere change in the shape of an organ will, of course, alter the existing tensions and occasion-

ally establish new ones. Furthermore, it is obvious that the growth, or change in form, of an organ not only affects the ultrastructure inside but affects the surrounding ground-substances as well. Imagine, for instance, the growth of a vesicular or tubular organ. It is easy to realize that by increasing in diameter it must create a steady tangential tension in the surrounding medium which, eventually, leads to the formation of concentric fibrous capsules and sheaths. A tubular organ growing in length and width is bound to exhibit both longitudinal and circular tensions on the surroundings which may result in a corresponding orientation of the surrounding tissues. Examples are found everywhere in the body.⁵ Carey (1922) has called attention to the fact that the arrangement of muscle tracts in the embryonic limb can be explained on the basis of tensional stresses exerted on the surrounding mesenchyme by the differential growth of the skeleton. A similar explanation was claimed for the arrangement of the muscular layers in the intestine (Carey, 1921). Considering all existing evidence, it seems that this idea is perfectly substantiated, as far as merely the spatial arrangement of the tissue is concerned. On the other hand, there has not yet been offered any convincing proof to show that the differentiation into muscle tissue is likewise determined by tensional strain. On the contrary, whereas there is some indication that stretch of some kind is essential for the maintenance, and maybe also for the differentiation of muscle fibers, the facts revealed by experimental embryology carry sufficient evidence to emphasize that the factors determining a cell to transform into a myoblast are of a more specific nature than mechanical stress can ever be.

From what we have stated above, it becomes clear that even the epithelial forerunners of organ formation, by

⁵ It is obvious that the tangential stretch which leads to the formation of a fibrous capsule around an organ is not always produced by increase in size of the organ, but may as well be due to a gradual contraction of the surrounding substance, as is the case in the formation of capsules around foreign bodies.

their moving, folding, stretching, swelling, and so on, must have a marked effect on the ultrastructure of the underlying ground substance, in later stages on the mesenchyme. It is however, hard to say whether this phenomenon is of significance in the process of organ formation or is just a transitory incident. Observations on the development of glands by Flint (1903) suggest that the epithelial formations may play some rôle in the organization of their supporting or surrounding stroma.

The fact that I have been dealing at some length with the effects of mechanical tension may give a wrong impression about the real share which those tensions take in organization. As a matter of fact we frequently find their importance overrated by many authors. Of course, we do not know very much about the other orienting forces besides tension which intervene in morphogenesis. But we know, at least, a little, and this may be outlined as follows: One non-tensional factor which can be assumed as certain to exhibit organizing activities by stamping ultrastructures in the ground-substance is the displacement of fluids, both by slow diffusion and by faster circulation. You all remember having seen brooks streaming over grass or weeds. You remember how the blades were oriented by the current, as if combed. This gives an illustration of how the circulation of liquids in the ground-substance causes the formation of an ultrastructure oriented along the stream lines. Steady diffusion potentials work, of course, in the same way. Undoubtedly, many of the whorl-like formations in organisms can be explained on this basis. I have frequently observed typical stream-lined structures in the semi-fluid regeneration blastema. The morphogenetic action of the displacement of fluids is, of course, most pronounced in those cases where there is excessive resorption of water during growth. Triepel (1911) was already aware of the fact that the fiber tracts of the connective tissue in a tadpole's tail mark those directions in which water from outside penetrated into the tail, and those along which it

shifted therein. Once aware of these factors one will probably detect them in many places. It is, for instance, very likely that a difference in the water content of different organs has effects of the kind described. To a certain extent, I was able to reproduce and analyze even in tissue culture the directive effect of fluid displacement. A growing tissue culture, by sucking fluid from the surrounding medium, establishes a steady diffusion gradient. If, now, two cultures are put in the same medium, their reciprocal suction will result in a movement of liquids along their line of connection. This movement results in the formation of a corresponding ultrastructure bridging the two growth centers, and the bridge becomes visible, as soon as the cells grow over it (Weiss, 1929). In the embryo, pictures of this kind, showing strands of cells forming straight connections between two centers of high formative activity are a familiar occurrence. If there is a mechanical barrier somewhere in the ground substance, currents from the surroundings will be deviated in a direction parallel to the wall. This causes the formation of stream-lined ultrastructures which sometimes can be easily mistaken for tensional structures. The transverse growth of a tendon regenerated along a silk thread inserted at right angles to the direction of the tendon stump, as found by Levy (1904), is apparently due rather to the flow of liquids along the thread than to tension, as the author was inclined to believe.

Another factor which during morphogenesis undoubtedly is involved in the formation of ultrastructures is the electric field. Electric fields may act either directly by orienting the polarized micellae or indirectly by causing cataphoretic effects. These latter consist in the establishment, in the capillary spaces, of an oriented migration of electrically charged particles along the lines of electric force, and this polar migration has, of course, the same orienting effect on the ground substance as a streaming fluid. Increased chemical activity in a circum-

scribed area leads to structural effects both by causing diffusion streams and electric potential differences. The chemical activity may furthermore alter the qualities of the ground substance itself, may cause dehydration and fibrillation or, on the other hand, by means of proteolytic enzymes, cause some liquefaction and destruction of existing structures. And if we try to survey all possibilities, the situation becomes so intricate that, at least, one obvious objection against our conception, namely, that it might be too simple, certainly can not be upheld.

As an example of how the principle of structural organization can be applied to special problems in embryology, I may briefly outline its explanatory value for the problem of nerve patterns, both peripheral and intracental. As Harrison (1910) has concluded from his tissue culture experiments, outgrowing nerve fibers show a similar affinity to solid structures, as do mesenchyme cells. So, there was good reason to believe that ultrastructures in the ground substance might be responsible for the orientation of the growth of the nerve fiber. This assumption is substantiated by the observation of Held (1909) who saw the first processes of the embryonic neuroblast extend along the extremely fine filaments of the mesostroma connecting medullary tube, myotomes, notochord and ectoderm. Thus, the facts support the idea that the immediate factors in the orientation of the nerve fiber are correspondingly oriented guiding structures. On the other hand, however, various other kinds of factors have been proved to influence the orientation of nerve fiber growth. The theory of neurotropism as a form of chemotaxis has been advanced by Cajal,⁶ electric fields have been claimed by Kappers (1927), Child (1921) and Ingvar (1920) and Detwiler (1926), finally, have clearly shown the attractive influence on the outgrowing nerves which is exerted by a developing organ rudiment. The principle of ultrastructural organization offers a common and uniform explanation for all these cases, by

⁶ Cf. Tello, F. Vortr. u. Aufs. u. Entwicklungsmech. H. 33, 1923.

assuming that neither the chemical nor the electrical agents act directly on the nerve fiber, but that both, primarily, produce ultrastructures in the ground substance which, secondarily, serve as guiding paths for the nerves; chemical centers by diffusion currents, electric centers by cataphoresis, both resulting in structures converging toward the respective centers. The occurrence of a peripheral nerve plexus in the limb region could be explained by assuming that there is some metastructural barrier arising from the meeting of two fields of activity, one being the developing limb bud, the other being the axial organs of the embryo. An interlacing of nerve fibers, corresponding to what in the organism occurs as plexus formation, could also be observed in the tissue culture in places where there was some sudden change in the mechanical condition of the medium.⁷ The formation of intracentral fiber tracts may obey similar rules. According to Kappers, Coghill (1929) and others, central fiber tracts always connect such parts of the brain as differentiate simultaneously. This reminds us exactly of the conditions of the experiment described above where a cell bridge connected two growing cultures. Furthermore, Bok's (1929) demonstration of the dependence of the internal architecture of the brain upon its curvature almost forces us to consider this architecture as trajectorial. These few remarks may suffice to show the applicability of the principle.

Having established and demonstrated the principle of ultrastructural organization its general bearings may now be pointed out. It is clear, at first glance, that the principle tends to replace a number of different agents acting at a distance which have been hitherto considered as orienting and organizing factors. If cells arrange themselves in definite directions this should not be explained any longer by specific attractions nor by trophic stimulation, but by the establishment of oriented pathways in the ground substances, these latter being the

⁷ P. Weiss—Unpublished results.

common playground of all kinds of forces released during development.⁸ Although the principle apparently applies only to moving cells, leaving the cause of their displacement unexplained, a consideration of the changes in surface tension relations, in distribution of liquids, etc., may possibly lead to an understanding of this latter point, too. A certain difficulty seems to arise, however, from cases where in one and the same organ or embryonic district one part shows definite orientation, while another part does not, or does, but differently; for instance, nerves running across muscles. It is, however, possible to account also for these cases if one keeps in mind two things: First, a possible specificity of the pathways; and second, what we may call the time factor. What specificity of the pathways means was expressed above when we said that differences in the surface tension relations at the solid-liquid interfaces of the medium may well explain why one type of protoplasm extends along the interfaces, whereas another does not. As to the time factor, the rôle of which in development has been especially emphasized by Goldschmidt (1927) and by Brandt (1928), we must be aware of the fact that the directive agents during embryogenesis are very unstable, most of them acting only temporarily, during a definite period. A center of higher developmental activity will, of course, act as an organizing agent on the ground substance only as long as its activity lasts. As soon as its activity decreases and other areas rise to increased activity, the previous ultrastructural pattern will gradually be replaced by a new pattern. Structures developed on the basis of the new pattern will, then, no longer show relations to the structures originated at an earlier time. In this respect the principle agrees with the general conception of tem-

⁸ There is evidence to show that not only the orientation and arrangement but also the shape of the cells is materially influenced by the organization of the ground substance. It might, therefore, well be that in the loose connective tissue the lamellar structure is not, as Laguesse believes (*Arch. de Biol.* 31, 173, 1921), the result of, but is the cause for the flat shape of the cells contained therein.

porary gradients of activity as conceived by Child (1929). Taking into account the typical sequence of developmental processes, the time pattern, so to speak, we recognize the developed organism as being, in some way, comparable to a photographic plate on which different pictures have been developed successively, and the difficulty mentioned above ceases to exist. One more thing, however, has to be assumed. That is, that the ultrastructures are plastic and labile enough to give way to new ones, if this is required by the situation. This point is the main reason why so much emphasis has to be laid on proclaiming ultrastructures and not manifest fibrillar structures as the general guiding principle. Ultrastructures, indeed, are plastic and reversible and thus conform to the requirements of embryogenesis; fibrillar structures, generally, are not. Fibrillar structures, too, are not altogether unmodifiable, but their breaking down seems to be too slow to keep pace with the rush of developmental changes. Since, however, ultrastructures tend to transform steadily into coarse fibrillar structures, provided they are not stirred up by continual interferences, a gradual loss of plasticity during development may be expected. The degree of plasticity found in any part of the embryo will, then, essentially depend upon how long an organizing factor has had time to act on the surroundings. After a certain duration of continuous uniform action, the ultrastructure will be found to be almost irreversibly transformed into a manifest structure no longer susceptible to new influences; such solidified structures, however, will, on the other hand, be able to serve, henceforth, according to their previously attained organization, as a typical guiding system, even if the original organizing factors do not persist or have changed in character. We will say of such a system that it has undergone "irreversible determination" and has changed from dependent differentiation to self-differentiation. This remark should, however, not be mistaken. I do not think we can expect that the general problem of deter-

mination could at some time be reduced to so simple a basis. Past experience has warned us of being too prompt in generalizing. So, instead of proclaiming the principle of ultrastructural organization as a universal clue for the problems of embryonic organization, we may content ourselves, for the time being, with the recognition that it is *one* of the principles or mechanisms of morphogenesis. Its best feature is that it opens innumerable ways for further experimental attack.

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ing interest, as distinguished from idle curiosity, grew. Then came the period of rapid growth and wide-spread utilization, as manufacturing methods became economical and efficient. This was followed by a period of slackening of interest in which the bicycle ceased to arouse curiosity and in which it became a toy or a strictly utilitarian means of locomotion. Meanwhile the first automobiles were making their appearance. They too passed through a fore-period of growth and manufacture. We have recently passed into the period of maximum growth of interest in and of manufacture of automobiles in this country and presently we shall pass into the last phase, into the period of complete practical adjustment in our use of this means of locomotion. Meanwhile, the aeroplane has come along. The development and maturation of this spectacular means of locomotion was prematurely hastened by the needs and interests of the Great War. Its problems are not yet fully solved and it is probably not yet known just what it may contribute permanently to man's help and comfort.

In a similar way, we could perhaps show that the history of every innovation, produced in the development of man, follows a similar course. The use of fire, the building of roads, the use of horses for travel, the development of stage coaches, of railroads, of plows, of looms, of typewriters, of telephones, and of hundreds of other comforts, utilities and practises of human life, past and present, have developed in a similar way. This even extends to the styles of clothing that we wear and to the minutiae of our personal habits. It is almost trite to say so because we are so accustomed to it, but there is always the fore-period, the period of maximum interest or change and the after-period of loss of interest.

When we analyze the history of developments of any kind we find that they all show a fore-period of preparation followed by another period of maximum activity succeeded by another period of rest and preparation for the next cycle. There is some kind of external stimulus

and internal accommodation that constitutes the fore-period; this is followed by a period of great activity until depletion in one of the necessary agents occurs, and then the process enters into the decay stage which may be the fore-period of a new cycle. The dimensions of a given cycle must be retained in discussing that cycle. Cycles within cycles occur here also to an almost unlimited degree of complexity. Within its own dimensions the characteristics of a given cycle are not disturbed by another or series of others of a larger or smaller scale. We shall discuss these things more fully below.

Growth of human knowledge an autocatalyzed process: Viewed in broad general terms the phenomena that we have just been discussing may be considered as education. Both the development of the innovation or invention in the minds of men and its transmission to others are autocatalyzed processes. We know when we come to think of it that education follows the autocatalytic curve. We find that our effective interest in any subject is slow of growth. It then comes to full maturity and intensity, and finally wanes unless fed by new view-points and material. Achievement is likewise autocatalytic. Men finish their work in one field and move to another if they wish to remain fruitful. Wilhelm Ostwald in his study of the biographies of great scientists, undertaken in order to learn the secret of their greatness, concluded that this slow initiation of achievement and gradual shifting of interest is a rather general trait of the greatest fruitfulness of creative genius and cites Helmholtz as a notable example. This he suggests may be due to the exhaustion of certain materials in some of the neurones, that are essential to the development of the autocatalysts, while in other neurones an unimpaired stock of these materials is still available, but unfortunately not transferable. But it is also possible that the fruitfulness of genius depends upon the presence of raw material, so to speak, in the environment. Just as an amoeba fails to grow in an environment containing no food, so genius may fail to grow

in the absence of its own peculiar "food." The nature of this "food," or stimulus to fruitfulness, varies with the nature of genius. And so it comes about that a change of activity or of surroundings acts like a renewal of youth. Men of genius are wise to use one or both of these methods to sustain their productivity.

As we grow older we learn to recognize this sort of fatigue or exhaustion for what it is and to discount the negative reactions that sometimes accompany it. It is so much more difficult to recognize it in great men, who have performed heavy labors, that it is beyond our ability to comprehend. T. B. Robertson in the preface to one of his books¹ describes an interesting instance of this sort. Bergson, in his "Creative Evolution," asserted his belief that the scientific method can never ascertain a solution of the riddle of development. Robertson's comment is in part as follows:

This assertion affords but one among very many contemporary instances of waning faith in the power of intellect; a reaction, perhaps against its overwhelming successes, which nevertheless continue without ceasing, and augment. . . . So little effort and we are now exhausted! So brief a period and we abandon the conflict! We declare ourselves impotent, because in a day we have not comprehended the ultimate significance of life! . . . M. Bergson has himself eloquently and convincingly dwelt upon the vast import of our understanding, and the argument which he advances is applicable no less to the more intangible advances of theoretical knowledge. Each successive acquirement enlarges our power to grasp more, and the progress of human knowledge is in fact autocatalysed.

We must pause here and emphasize the fact that the autocatalytic growth of knowledge either in the mind of man or in the tradition of the race is not simpler than an autocatalyzed chemical reaction, where the proper autocatalyst is not the sole prerequisite. There must be a suitable substrate, a suitable medium and a suitable energy environment. Failure of any of these will make the autocatalyst ineffective. As educated men and as educators we generally fail to appreciate either in ourselves

¹ "The Chemical Basis of Growth and Senescence," Lippincott Company, 1923.

or in others the complexity of the processes that we are trying to set up.

If the workings of the mind and if the growth of knowledge, in the individual as well as in the race, are indeed autocatalyzed processes, as we have been saying and as many think, then we as educated men have a very deep and personal lesson to learn, both in courage for ourselves and in tolerance for others. When we have finally by a slow autocatalyzed process reached the burning conviction that we are in the possession of new or valuable truth we must have the courage and patience to set up and keep going the autocatalyzed process of learning in our colleagues, until they too see the truth of our new knowledge.

In this connection we are reminded of the familiar story of Mendel. One of his friends sympathized with him twenty years after the publication of his famous work on heredity in sweet peas, because his work was not yet recognized. Mendel comforted his friend by saying, "My time will come." He knew of the long lag in learning, of the slow growth of the autocatalyst involved.

Autocatalysis in history: In recent years certain scholarly historians have pointed out the similarity of the curve representing the development, fruition and decline of races, of civilizations, of political institutions, etc., and the autocatalytic curve. In doing this they have been led to a rather pessimistic outlook from a consideration of the last phase of the simple autocatalysis curve, i.e., the period of depletion of the substrate. When the autocatalysis is considered as a unit, as a finished thing or process, the last phase of a cycle constitutes a very unsatisfactory stage. Because of the great progress or change taking place in the preceding phase it comes to be viewed as a dénouement period, the "sucked-lemon" period. It is my opinion that these writers have carried the physico-chemical analogy too far or else not far enough. They have failed to note that any autocatalysis curve of a chemical process represents only a segment of

the life history of the bit of matter in question. As we have seen above, a suitable change is pretty likely to occur that will give rise to a new cycle and so the disappointing part of the curve becomes the fore-period of a new cycle.² Of course the new cycle can not be the same cycle as the one just past. Moreover, in terms of the duration of human life, it may be that we can not live to see the next cycle completed, but we need not for that reason fall into tears by the shores of "time," thinking that life is of no further interest because the last breaker has rolled in. It is true that for us it may be the last of its kind, but we may be comforted because even as we watch it finish its course the forces of the "sea" are being accumulated as the fore-period of the next great wave. And so it seems to be with the "breakers" or cycles of history.

The ancients had no such grounds for comfort in this respect as we have. If we had stood with the people of Greece and had seen that beautiful wave of democratic civilization break on the shores of time and slip back into the seas we might have wept for grief and inability to see the new great wave of democratic civilization in the state of formation. We, however, happen to live in a time in which another great wave is emerging. Perhaps none of us can yet say whether this cycle of progress in democracy will sweep the world or whether even now a greater broader wave is forming. We know that the democracy of Greece was made possible by slave labor. In the cycle now approaching completion slavery has largely been replaced by mechanical power. The age of machinery has raised the crest of the wave to a higher

² I first encountered such ideas somewhere in the historical writings of Henry Adams. Later of course in full force in Oswald Spengler's "The Decline of the West" and also in K. C. Schneider's "Die Periodizität des Lebens und der Kultur" (1926). It is beyond my capacity to add anything to the discussion of the problem of the cyclic nature of history beyond the simple suggestion of developing a more unified view and of seeing things in "scale." The work of these writers would therefore constitute authoritative discussions of certain cycles or even "octaves" of the great spectrum of life that we are trying to visualize.

level than it had attained before. Without going into further detail it is already evident that the present cycle is vaster in its scope, since it aims to include all men and not just a selected group. It is therefore quite unlike the former one and if it fails to sweep the world we shall have other grounds for disappointment than those experienced by the Greeks. The two could not be interchanged in time.

These cycles of progress have their proper order and sequence and this is the ordered sequence of musical notes in their octaves and that of spectral series. Many historians have had an intuitive sense of this ordered quality of the continuity of history, and this quality is distorted by a restricted use of the autocatalysis curve. To view the various aspects of civilization, or the process as a whole as an autocatalyzed process is apparently justified, but to leave out the principle of continuity by emphasizing the single cycle gives rise to pessimism. Such a pessimism contradicts our intuitive sense of continuity, of progressive evolution. This unreasoned and unreasoning sense of moving forward that possesses us is satisfied to work and rest alternately, as is provided for in this spectral picture of life and history.

Many writers have more or less intuitively realized this peculiar progressive continuity. Recently George Sarton expressed it as follows:

During the course of my studies of human progress, I have often had the impression that everything happens as if mankind was working in shifts. The accomplishment of its essential task is so hard that periods of creation are often followed by periods of fallowness.³

Autocatalysis of the evolution of Man: If we consider only the lower portion of the autocatalysis curve we have in a clearly recognizable form a so-called hysteresis curve, i.e., there is a lag behind the calculated response, when we are looking for a linear response. Without attempting to review or discuss hysteresis in its usual ap-

³ "Introduction to the History of Science," Vol. I, p. 443, Williams and Wilkins.

plication, we may at once consider what may be called the hysteresis of history, but which we now see to be the autocatalysis of the evolution of man.

In the course of man's evolution the most interesting and unique activity in which he has indulged is his comprehensive attempt to conquer his environment. Certainly there are but few living organisms that have made achievements comparable with those of man in this respect. Insects have much to their credit, in their adaptation of materials to their needs, but theirs is no longer a progressive tradition, for a given species, as in the case of man. The earliest knowledge that we have of man's conquest of his environment depends on the elaboration of crude stone implements. By the presence of such relics the relative age of human remains can be determined. By those relics it is indicated that perhaps a half million years ago man had made more progress in a voluntary conquest of his environment than any mammal has made up to the present. There is no way of knowing how these men discovered that by attaching a stone to the end of a club they could strengthen the power of their arms, but it is clear that such an initial step as this was more significant in man's development than all that follows. Such was the great new idea, the germ, so to speak, of a new autocatalyst that was to transform the whole nature of man and his attitude toward the universe.

It has been suggested that it took perhaps four hundred thousand years for this new idea to grow and ripen enough so that man made marked improvements upon these crude beginnings and then the Paleolithic period of his history becomes the Neolithic period, according to our terminology. During this long dark period opposition to the acceptance of the "new fangled" ways of fighting, with hammers, for instance, was only slowly overcome as tribes saw themselves decimated because of their unwillingness to accept and use stone hammers. By Neolithic time innovations took place more readily and man was using this new conscious way of improving

his life with more facility. Handicraft had a high place in social esteem, because it represents a skilful harnessing of the brain and the hand. Because of improved interest and skill progress during this period was more and more rapid so that after perhaps another one hundred thousand years we come to the invention of the steam engine. For some time before this man had been approaching a new type of conquest—a conquest of power—and the steam engine represented the fruition of this movement. With this invention a new age of still more rapid progress, in the conquest of our environment, was ushered in and it is in this age of headlong advance in which we are now living.

If we attempt to make a diagram representing the above facts and plot time against the amount of change or progress it is evident that we have the fore-period and the first part of the fruition period of the autocatalysis curve. If we pause now to consider what the remainder of the fruition period and the final or completion stage may bring to man we are silent. It is not likely that any one can predict in broad terms what the remainder of man's history will be, when viewed as a whole in these terms. If we view history as an autocatalytic process it follows that the change or progress occurs as the result and in answer to some external tension or stimulus. But the system in turn must contain within it the capacity to respond. What can be the nature of this stimulus and what can be the latent form of the response contained within the system. We are quite accustomed to visualizing some aspects of the two halves of this situation in the simpler forms in which it occurs in physics and chemistry, but here these pictures are inadequate. We must remain satisfied with a general picture. We see this earth of ours and all that it contains lying like a mass of molecular dimensions in an energy field of unknown qualities, so far as their final effect and influence are concerned. We feel a kind of cosmic heat warming us up toward efforts of development and achievement in which

the first steps at least are taken in darkness and uncertainty. When shall we achieve a clearer sight in these fields? Or is the scientific method in some degree the organ of sight for the mind as the eye is the organ of sight for the body?

At first it seemed that this is the longest autocatalysis in the known history of man that we could put our fingers on. When, however, we remember that man in his individual development undergoes a physical, intellectual and spiritual development and that the length of the autocatalysis curves for these increases in length in the order mentioned, the possibility of a longer cycle of development becomes evident. The evolutionary development of man's physical nature lies in the unknown past, the development of his intellectual nature as a race is in the state of active development. Where is the evolutionary development of his spiritual nature as a race? If the analogy holds will it not have a longer "wave length" in our historical or evolutionary spectrum than either his physical or intellectual evolution? Could we not expect that the emergence of man's intellectual nature should be accompanied by the early stages of his spiritual development? When we think of it in this way we get a clearer insight into the significance of man's persistent interest in his own origin and destiny and in his ultimate relation to the universe.

Such vague speculations and yearnings are usually lumped together and called religion. We see sporadic leaders of such interests rise and fall; they have experienced some power and insight in themselves and have tried to develop the autocatalyst in their neighbors and the record shows us that they have done so with measurable success. Consequently, during the past four or five thousand years there has been an observable increase of interest and of effective usefulness of a spiritual autocatalyst. However, even the most optimistic could scarcely assert that man has as a species entered into the period of full fruition in this strange realm. But none

will gainsay, if we have read the record correctly, that if and when man does enter into the period of fruition of this long autocatalyzed process, in which at best he is still in the fore-period or period of preparation of the autocatalyst, he will undergo as strange and marvelous a transformation as that of the savage carrying a club with a rock tied on the end of it, being transformed into the highest product of man's present development. In respect to the development of his religious or spiritual nature man is somewhere in the Paleolithic period of his development. The present strife and struggle among the various forms of ethical religion represents the growth and perfection of the autocatalyst, an attempt to pass from the Paleolithic to the Neolithic stage of spiritual development.

Résumé: In this section we have extended the description of phenomena that follow the "S" curve resembling the autocatalytic curve. We have always found the "adaptation" associated with a "mutation." We have seen that when one cycle of this sort succeeds another they are different in some respect. We found such autocatalyses to be linked and interwoven, just as the various "wave lengths" of light are interwoven in sunlight. Although we could have elaborated the description of this "spectrum of life" in much greater detail and could have cited many other processes that follow this course, nothing significant would be contributed in this way. We are now interested to see, in the remaining section of this essay, if it is possible to find any basis upon which the behavior of such diversified systems can be visualized so as to give rise to this "S" curve. We can not hope to explain the long chain of events, but it may be possible, so to speak, to string them, as it were, on one connecting thread.

THE GENERAL BEARINGS OF RECENT RESEARCH IN OENOTHERA¹

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THE first period of genetic research was largely devoted to a rapid accumulation of data regarding many plants and animals by a relatively few investigators. The domestic or semi-domestic animals and plants particularly came in for early examination, many of the classical cases of Mendelian inheritance in rats and mice, fowls, sheep and cattle, maize, wheat, *Lathyrus*, *Primula*, *Antirrhinum* and *Capsella* dating from this period. Bateson, with his colleagues, became the world leader in the study of Mendelian behavior.

Already, however, in the pre-Mendelian era, the genius of de Vries had developed methods of pedigree breeding and amassed a large body of what we should now call genetic results. The genetics of *Oenothera* thus began a decade or two earlier than that of other plants, with the exception of Mendel's original experiments with garden peas. In the last two decades, under the guidance of Morgan, *Drosophila* has loomed large in animal genetics and has served for the elucidation of such fundamental discoveries as the correspondence between the number of linkage groups and the haploid number of chromosomes. When this principle was confirmed by Punnett for *Lathyrus* it clearly became of general validity. The phenomena of non-disjunction, trisomics and polyploids also appeared incidentally in *Drosophila*. Thus it has come about that *Oenothera* among plants, and *Drosophila* among animals, have longer and more complicated genetic histories than any other organisms.

Apart from the fact that cytological and genetical observations and principles have become more and more intimately interwoven throughout the whole period since

¹ Paper presented at the Sixth International Congress of Genetics, Ithaca, N. Y., August 29, 1932.

the beginning of the century, another conspicuous movement in recent years has been the phylogenetic study of particular genera, especially of plants, on a cytogenetic basis. Sumner's investigations of *Peromyscus*, its geographic sub-species and varieties, are a notable case on the animal side, in which the principles of evolution rather than the actual phylogeny were mainly considered. Thus, after a period in which genetical investigators were almost inclined to look askance at the discussion of evolutionary problems, the experimental study of phylogeny and evolution, through the investigation of the chromosomes, the hereditary genes and the systematic relationships of a group of forms, has come to the fore in various genera such as *Nicotiana*, *Crepis* and *Triticum*. By such means will genetics come into closer touch with the general problems of evolution. It seems clear that in the near future many intensive evolutionary investigations will be carried out involving the combined and co-ordinated study of the cytology, genetics, bionomics and geographical distribution of a circumscribed group and leading to a fuller understanding not only of the lines along which their phylogeny has taken place but also of the environmental conditions and the internal developmental factors which have been most effective in connection with their evolution. At the present time cytology is initiating such studies in many groups by comparative investigations of their chromosome numbers and morphology.

In *Oenothera* much has now been done which can contribute from many angles to a study of the phylogeny of the genus. I propose to refer to this subject later, but I wish first to make brief reference to the earlier genetic history of *Oenothera*. It is so well known that I need scarcely remind you that the first work on mutational changes in chromosome numbers was done with *Oenothera*. The classic case of *Oenothera gigas* was the first genetic tetraploid, with which the vast modern study of polyploidy began. Similarly, the first trisomic was *Oenothera lata*, and the first triploid *O. semigigas*.

Again, in *O. rubrinervis* the first case of non-disjunction in any organism was discovered. These developments take us up to 1915.² They provided an explanation of the manner of origin of many of the well-known mutations originally described in the experiments of de Vries; and cases of similar character are now known in many other genera of plants and animals. Curiously enough, however, although polyploidy and to some extent non-disjunction are undoubtedly of phylogenetic significance in connection with the evolution of many plant genera and families, yet they have apparently played no part in *Oenothera*, where they were discovered, since all the known wild species of *Oenothera* agree in having 14 chromosomes. Evidently in this genus some other process has been at work, and we may expect to find it in connection with the chromosome catenation which is so wide-spread in the genus.

In the post-war period the study of fixed chromosome linkages or catenation began with the paper of Cleland (1922) on *O. franciscana*. This has now become a field of very active research, but many uncertain interpretations are involved, some of which will be discussed later. We now know that the great majority of species, hybrids and mutations of *Oenothera* show more or less complete catenation of their chromosomes during the period immediately preceding the heterotypic metaphase in which chromosome reduction takes place. But the condition, which appeared for some time to be unique in *Oenothera*, is now known to occur also, in *Rhoeo*, *Datura*, *Pisum*, *Aucuba*, *Campanula*, *Humulus*, *Avena*, *Zea*, *Rosa*, *Polemonium*, *Hypericum*, *Rumex*, *Briza* and *Anthoxanthum*, which are representatives of a series of Monocot and Dicot families. But only in *Rhoeo* and *Hypericum* has the complete catenation which is so characteristic of *Oenothera* been found.

That catenation has played an important part in the evolution of the genus seems clear, but we have at present no means of knowing why it has become so para-

² Gates, "The Mutation Factor in Evolution."

mount in *Oenothera* while in many other genera it has little or no significance. I think, however, we may understand the situation better when we realize that catenation helps to perpetuate a heterozygous condition, and that the concomitant hybrid vigor will give a marked selective advantage to catenated over uncatenated forms. It has been shown experimentally that a ring of 4 or a ring of 6 can arise by crossing two homozygous *Oenotheras* each having 7 free pairs of chromosomes. In the presence of balanced lethals the catenated hybrid form will persist, while the homozygous recombinations will entirely fail to appear; but even without such lethals there is every evidence that the more homozygous recombinations with less catenation will fail to survive under natural selection because of their markedly decreased hybrid vigor. Thus the catenation of chromosomes, once begun in a hybrid form, would tend to increase to the maximum—a ring of 14. The forms with less catenation, such as half-mutants like *O. rubrinervis*, which may be supposed to arise through segmental interchange between chromosomes of a ring belonging to different complexes, will have less hybrid vigor, and may therefore be expected to be snuffed out in competition with the more heterozygous forms from which they are derived. The same will be true generally of completely homozygous segregates such as *deserens* and *blandina*. Thus hybridization appears to be a necessity for the development of maximum catenation such as *Oenothera* shows, and in this respect it must have played a fundamental rôle in the evolution of the genus.

Elsewhere I have discussed (Gates, 1933) the phylogeny of the genus *Oenothera* mainly from the systematic and geographical points of view. Here it is only necessary to consider the various genetic principles involved. Investigators of the genus are agreed that the large-flowered southern species are primitive, and it seems clear that these were at first confined to Central America and Mexico. As the ice retreated at the end of the Pleistocene they moved northwards and gradually spread

over the continent. Since they have no special method of seed dispersal, it is reasonable to suppose that their spread was slow and that there was ample time for evolutionary differentiation to take place in localized areas.

Now there appears to be a definite relation in *Oenothera* between flower-size and latitude, the small-flowered species being massed in the higher latitudes while the large-flowered forms are found mainly in the South. There is considerable evidence (chiefly unpublished, see Gates, 1932) that the species with small petals have been derived by a series of dominant mutations from those with larger flowers as this northward movement was taking place. It seems that genes for smaller flower-size have appeared independently in different lines of descent, *e.g.*, on the Atlantic and the Pacific coasts. In general, decrease in length of style goes hand in hand with diminishing flower-size, so that the large-flowered species have long styles and are open-pollinated while the small-flowered species are usually self-pollinated. Although there are exceptions, it is quite probable that the same gene which decreases the length of petal has a similar effect on other parts of the flower, including length of style. Statistical evidence derived from large numbers of measurements of petal length in various interspecific *Oenothera* hybrids leads to the conclusion that some of the small-flowered species, but not all, contain several genetic factors for decrement in petal length. It is also found that these size genes are not all of equal value, some producing a larger effect than others. Further evidence is furnished by the fact that in *O. novae-scotiae*, a small-flowered species with complete catenation, one of the complexes contains factors for a considerably larger flower-size than the phenotype of the species (see Gates and Catcheside, 1932).

Since decreasing flower-size leads to self-pollination, it is probable that hybridization was of more importance and of more frequent occurrence in the early history of the genus than in its later development. The bionomics of the small-flowered species are altered in several other

respects. Having smaller flowers, they save considerable energy in the production of calyx and corolla. The anthers are also much shorter and produce a relatively small quantity of pollen—again a considerable saving of energy. Further, notwithstanding the smaller quantity of pollen, they are much more efficient seed-producers, as any one knows who has grown the small-flowered and large-flowered species side by side. The former will usually show a complete setting of capsules filled with seeds, because the stigma of every flower is already pollinated before it opens (except at the end of the season), while in the large-flowered forms many flowers have failed of pollination through lack of insect visits, and the amount of seed production is usually much less. As a seed-producing mechanism the small-flowered species therefore have a marked advantage over those with large flowers; and since they are permanent cryptohybrids owing to the chromosome catenation, they do not suffer from homozygosis although they are generally inbred to the maximum degree. All these considerations help us to understand why there is a general diminution of flower-size with increasing latitude, and why the forms with smaller flowers occur generally in the areas most recently occupied in the northward migration. It would be interesting to know whether genes for flower-size have played a similar rôle in any other genus of flowering plants.

If we consider now the evolutionary factors which have been concerned in the phylogeny of the genus *Oenothera*, four at least may be mentioned: (1) gene mutation, (2) hybridization, already considered in certain aspects, (3) catenation, (4) segmental interchange of chromosomes. With the exception of (1), these factors or conditions are intimately associated with each other. It appears clear that gene mutations are as necessary to account for the evolution of *Oenothera* as of other plant and animal genera, but it would appear that they are usually masked by the existence of complexes, which in turn seem to depend upon catenation of the chromosomes.

The known gene mutations are *brevistylis* and genes for dwarfing discovered by de Vries in *O. Lamarckiana* and *O. gigas*; *rubricalyx*, the only known dominant mutation; and those which Shull has been particularly successful in obtaining from *Lamarckiana*, namely, *supplena*, *vetaurea* and *bullata* (Shull, 1925, 1926, 1928); also *acutifolia* recently described by Brittingham (1931).

The fact that, according to Shull, these fall into three separate linkage groups in a species having only one free pair of chromosomes, is difficult to reconcile with the apparent absence of gene mutations from other species with high catenation. If *O. Lamarckiana* can show unlinked gene mutations, why do not other related species having high catenation show free Mendelian segregation when crossed? The evidence that they do not is so clear, both from double reciprocal hybrids and from other systems of breeding, that we are forced to conclude that linkage of chromosomes is involved in their failure to appear. Shull endeavors to solve the difficulty, so far as *Lamarckiana* is concerned, by assuming that the position of the chromosomes in a ring is not fixed in the sense that chromosomes of each complex always alternate with each other. He assumes that for each pair the arrangement in the ring may be AB or BA, which would give the effect of free segregation for each pair which had this degree of freedom. There are serious difficulties with such a hypothesis in *Lamarckiana* itself, but the difficulties in explaining thus the hybrid behavior of catenated species generally are so great that it appears necessary to abandon the hypothesis. On the other hand, on the hypothesis that the chromosomes occupy fixed positions in the ring, chromosome catenation will explain the general phenomena of genetic linkage so characteristic of *Oenothera* hybrids.

In the earlier papers on catenation the configuration was regarded as practically constant for each species, hybrid or mutation; but almost from the first occasional exceptions were recorded, and in *O. Agari*, a member of the subgenus *Raimannia*, much variation in configura-

tion was observed (Sheffield, 1927) although the species breeds strikingly true. Recent papers (*e.g.*, Illick, 1929) show a certain amount of variation, two or three configurations appearing in the pollen mother-cells of a particular phenotype. The most remarkable range of catenation appears, however, in *O. rubricalyx* \times *eriensis*, recently worked out in my laboratory (Hedayetullah, 1932). Most of the hybrids from this cross die in the seedling stage, but the few survivors show a catenation which is frequently a ring of ten and a ring of four. More numerous pollen mother-cells showed a chain of 8+ chain of 4+ chain of 2. But in any of these groups a ring could replace a chain or *vice versa*. A single cell contained a ring of 14 and several a chain of 14, and other arrangements were observed as well.

This brings us to the interpretation of the cytological nature of chromosome linkage. It is known to arise through crossing in *Oenothera*, and it is reasonable to assume that in *Datura* and *Pisum* and probably in *Oenothera* it has arisen through segmental interchange. A favorite hypothesis in recent papers has been that it always results from segmental interchange between non-homologous chromosomes, with terminalization of the resulting chiasmata. This hypothesis has much to be said for it, yet it encounters serious difficulties as a general explanation. The question of telosynapsis or parasynapsis is also clearly involved. For over twenty years *Oenothera* was regarded as an unshakable case of telosynapsis. Then efforts were made, especially by Darlington (1931b), to develop a hypothesis which would plausibly account for both parasynapsis and catenation by the terminalization of chiasmata. In a recent paper (Gates and Goodwin, 1931) in which a cytological study of meiosis in *O. desertens* and *O. blandina* was made, some evidence in favor of a parasynaptic interpretation was obtained in these two forms which show no catenation. It was also shown that where seven pairs of chromosomes are present there is no continuous spireme preceding diakinesis, but an appearance of such is produced by

the interlocking of ring pairs before they are condensed into the definitive pairs of chromosomes.

The theory of terminalization of chiasmata, however, has always been in a somewhat precarious state so far as *Oenothera* is concerned, from lack of indubitable observational evidence. The recent results of Hedayetullah (1932) on a ring-forming *Oenothera* hybrid are practically impossible to interpret on any but a telosynaptic basis. He uses the argument in an early paper of mine (Gates, 1908) that the chromosomes in a ring or chain must represent individuals, and not the lateral joined threads corresponding to two chromosomes and transversely segmented. But he also shows that the double appearance of the chromosomes in diakinesis is due to the presence of two chromonemata. He has demonstrated (Hedayetullah, 1931) in *Narcissus* root-tips that the chromosomes are double structures throughout the mitotic cycle. Hence it is natural to interpret the two threads visible in *Oenothera* chromosomes at diakinesis as the two chromonemata of a somatic chromosome. This is also contrary to Darlington's (1931a) hypothesis that meiosis differs from mitosis in that the contraction of the chromosomes in the heterotypic prophase has anticipated the division of each chromosome into two threads. Hedayetullah shows in fact in *Narcissus* that the chromosomes split not in prophase but in metaphase, the two halves remaining in close juxtaposition until they separate in the succeeding metaphase.

The chromosome connections in *Oenothera* Hedayetullah regards as due not to terminalization of chiasmata but to portions of the linin thread being laid bare by the aggregation of the chromatin about certain centers in a spireme which is continuous for each ring or chain, thus forming the linked chromosomes. This point of view appears to be supported by the observation of Sheffield (1927) that in *O. eriensis* the connections between the chromosomes in the ring of 14 are usually noticeably longer than in other species. This peculiarity is also found to be inherited in some at least of the hybrids of

O. eriensis (unpublished). There is no room for an explanation of these facts in the simple idea that the connections arise through the terminalization of chiasmata.

With regard to the relation between catenation and segmental interchange, the following remarks seem pertinent. That the transposition of a portion of a chromosome can occur in nature seems clearly proved from the genetic comparison of *Drosophila melanogaster* and *D. simulans*. The evidence shows a similar transposition to have taken place in certain strains of *Datura Stramonium* and *Pisum sativum*. It is reasonable to suppose that such changes occurred in the early history of *Oenothera*. It will be remembered that these early southern forms apparently had large flowers and were therefore much cross-pollinated. Also in their dispersal northwards new types which had long been separated and had become differentiated by accumulated gene mutations would be brought together again. If segmental interchange had occurred in the meantime, then catenation would arise in these interspecific hybrids; and in those crosses in which the parents had already acquired small flowers through one or more mutations in this series, the hybrids would go on being inbred. If they had not acquired balanced lethals they would segregate more homozygous types with less hybrid vigor—types which would generally disappear in competition. Once balanced lethals had been acquired, the interspecific hybrids would produce only one or two types, which would continue to breed true.³ That hybrids between species with complete catenation frequently also show the same condition—a ring of 14—we have recently found from several instances (see Gates and Catcheside, 1932).

In addition to the types of interspecific differentiation already mentioned, it must be remembered that cytoplasmic differences have also appeared, although we

³ Both Muller (1930) and Haldane (1932) have concluded that an *Oenothera*-like condition with balanced lethals would only be likely to arise in an inbred organism. From our point of view it would therefore arise after the mutations for small flowers had appeared.

have no present knowledge as to how they have arisen. It must also be borne in mind that interspecific *Oenothera* hybrids are usually intermediate or in some sense a blend between the two parent species.

It might be supposed from these facts that there is practically no limit to the number of "new species" which could be formed by bringing into contact unrelated species with complete catenation. That this is not the case is shown, however, by several lines of experimental evidence. Besides the well known phenomenon of blending and breeding true in interspecific *Oenothera* hybrids, a contrary series of segregation phenomena is coming to light. This consists of (a) chromosome rearrangement so as simultaneously to reduce catenation and heterosis; (b) reversion to the grand-parental species in double reciprocal crosses, as originally shown by de Vries, (c) elimination of certain complex combinations through lethal conditions or, (d) through certation, *i.e.*, competition of the pollen tubes or competition between megaspore complexes. There is evidence that one type of egg is more or less completely excluded in this way in many crosses.

These phenomena enable us to understand why the small-flowered *Oenotheras* with complete catenation do not form a continuous series, such as one might otherwise expect to arise from occasional crosses occurring in nature.

We may conclude generally that segmental interchange played its part early in the history of the genus, accompanied by intercrossing. Then followed the northward movement. With the appearance of small flowers, came an era of inbreeding accompanied by increasing catenation and therefore perpetuation of the heterozygous conditions, with occasional outcrosses some of which would produce new and constant types. Hybrid vigor has led to ever-increasing heterozygosis of the surviving forms, tending at the same time to increase catenation to a maximum and eliminate the more homozygous derivatives. In the meantime, gene mutations have supplied the neces-

sary material for species differentiation. It seems obvious that hybridization or segmental interchange alone could not do this. There are many points, however, in the relation between gene mutations and segmental interchange which require elucidation, but I shall not consider them here. Can chromosomes become non-homologous through sufficient gene mutations, or is some other process essential? And what is the real basis of chromosome individuality, which retains their unity notwithstanding crossing over and the various forms of translocation? When we know the basis of the unity of the chromosome we may be able to answer these questions.

It is obvious that our study of the phylogeny of the genus *Oenothera* has only begun. To test the tentative views here put forward we need a very large body of observations, systematic, cytological, genetical and geographical, on the host of highly diversified forms which exist. In order to bring these under experimental study, I suggest that oenotherologists form a concerted scheme for collecting seeds from particular areas so as to obtain an understanding of the *Oenotheras* which occupy any region. I have been studying and describing forms from different parts of Canada for some years. Another group has been at work in California, others in Michigan, New Jersey and elsewhere. Might not the territory be mapped out in such a way that each group would be responsible for collecting the seeds, through local botanists or otherwise, from each area. In this way the characters and distribution of each type, regarding which we at present know very little in detail, could ultimately be determined. No doubt it would be necessary for each group of workers to specialize in particular local areas over a period of years; but ultimately by such means we should have accumulated a body of genetic, cytological and systematic data which would make possible a survey of the phylogeny of the genus attainable in no other way.

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SOME PECULIAR RELATIONSHIPS BETWEEN ECTOPARASITES AND THEIR HOSTS¹

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A STUDY of the relationships of parasites to their hosts may be fruitful in a number of important ways. And in particular such a study is of value in judging relationships of the hosts themselves, their geographical distribution, their palaeogeography, and the phylogeny of both hosts and parasites. The reason why a study of the phylogeny of a parasitic group may throw much light upon the phylogeny of the hosts of the group is because parasites (especially fixed parasites) usually evolve with their hosts. Where they do, this type of evolution is termed "paralleled" phylogeny. But parasites do not always remain exclusively with the hosts of a single species, they may spread to other hosts which offer in or upon their bodies a similar "ecological habitat." Because of this ability of parasites occasionally to spread to new hosts their study is of great importance to that of geographical distribution and palaeogeography.

The object of this contribution is not to discuss the details, the scope, or the general results of the host-parasite method of study but to present certain new facts and to summarize and interpret certain old ones that have come to the writer's attention during recent years while studying ectoparasites. It is hoped that this contribution may have its justification in calling to the attention of biologists the opportunities for utilizing the host-parasite method of study, and in stimulating a desire for further cooperation on the part of students of the hosts and those who study their parasites.

HOST PREFERENCES

The sucking lice, or Anoplura, appear to have a high degree of host specificity. Yet recent experiments indi-

¹ Paper read at joint meeting of American Society of Parasitologists and American Society of Zoologists, New Orleans, December 29, 1931.

cate that when impelled by hunger and the desire for warmth they will accept as host the mammal available.

In testing this point lice, *Pediculus* (*Parapediculus*) *atelophilus* Ewing, of the gray spider monkey, *Ateles geoffroyi*, were taken from their live host at the National Zoological Park, and offered a feeding upon the writer's arm. Far from being repelled by such a foreign host, they readily took to him, and two adult females fed to repletion. This they did in exactly the same manner as does the body louse of man, *Pediculus humanus corporis* Degeer. Following their engorgement they were kept in what appeared to be almost ideal conditions in a breeding cell next to the body, yet they both died in a few hours.

In another experiment lice, *Pedicinus* sp., from a sick baboonlike monkey, *Magus* sp., were transferred to the arm. These also readily took to the human host, and several began pricking the skin. Two of them, an adult female and a nymph, engorged to repletion. When these two were subsequently kept under what appeared to be good living conditions both died in a few hours.

Monkeys and man are primates belonging to the same order of mammals, and having, as is well known, blood and other physiological characteristics of a somewhat similar nature, yet when sucking lice are taken from hosts of other orders and placed on man, results similar to those just described may follow. Thus specimens of the sucking louse of the dog, *Linognathus piliferus* (Burmeister), taken from their canine host were placed on man, and out of 13 individuals used in two trials 3 fed within a short time. One of these, an adult female, fed for 45 minutes and then was removed to a breeding cell. She soon died, the blood appearing to be only partly digested.

These observations on the experimental transfer of sucking lice from their normal to a foreign host are not only of interest as appearing to indicate a lack of acuteness of the senses of smell and taste in these ectoparasites, but they are of significance in showing that sucking lice will accept foreign hosts. In nature they probably

at times make transfers upon contact of foreign with favored host species.

This tendency to accept foreign hosts may explain the presence in America on spider monkeys, species of *Ateles*, of lice that are so similar to the pediculid lice of man that for many years authorities believed that they were identical. All these monkey lice differ in a few minor characters from those found on man. Yet, knowing the tremendous gap in the natural affinities between man and spider monkeys, who would insist that the presence of these pediculid lice on the latter indicates a paralleled phylogeny of hosts and parasites? The only logical conclusion, from the phylogenetic standpoint, is that the spider monkeys obtained their lice for the first time following the arrival in tropical America of the American Indian. That the Indians were hosts of a *Pediculus* is shown by the presence of these lice on prehistoric Indian mummies.

INTERPRETING HOST PHYLOGENY FROM A STUDY OF THE RELATIONSHIPS OF THEIR PARASITES

Professor V. L. Kellogg and the late Professor L. Harrison have been ardent students of the phylogeny of the biting lice and each has pointed out how the evidence obtained from a study of the relationships of the lice may affect, pro and con, the interpretations of the phylogeny of the hosts. Harrison (2), in addressing the Linnean Society of New South Wales, in 1928, said:

The ostriches of Africa and the rheas or nandus of South America are commonly supposed by ornithologists to have arisen from quite distinct stocks. But their lice are so similar, and so different from all other bird-lice, that these must have evolved from a common ancestor, and so also must the birds themselves. Evidence derived from lice is confirmed by cestode and nematode parasites of the two groups of birds. Thus a phylogenetic relationship may be established by means of parasites. Equally, a supposed relationship may be refuted. Their lice prove that the penguins are in no way related to any northern group of aquatic birds, but belong in an ancient complex which includes the tinamous, fowls and pigeons; that the kiwis of New Zealand are modified rails, and not struthious birds at all; that the tropic-birds are not steganopodes but terns, and so on.

Without wishing to endorse all these conclusions of Harrison the writer would like to add that the ostriches of Africa and the rheas of South America have the same mite parasite, *Eupterolichus bicaudatus* (Gervais), and that this mite is not known from any other birds.

THE LICE OF COWBIRDS

The differences between related species of biting lice, or Mallophaga, are believed to have arisen largely because of isolation on certain very similar hosts. Such isolation prevents interbreeding of varieties of species or other closely related forms, and allows them to accumulate minute inheritable increments in any direction.

But what are the factors that support this isolation of species and under what conditions are they rendered inoperative? Kellogg (4), as long ago as 1896, emphasized particularly the fact that in the case of non-gregarious wild birds few contacts are made even between individuals of the same host species and are seldom made between individuals of different species. Among individuals of a single species contact is brought about between adults chiefly during mating, and between parents and young during the nesting period, and between young individuals while in the same nest. To what degree this lack of contact operates to restrict a species might be tested by transferring specimens of a louse species from their natural host or hosts to those of other species. This has already been done in a very limited way, yet with the lice of certain birds it is hardly necessary, for in nature we find the experiment being constantly performed for us and under conditions even better than man can devise and on a scale his ambition would hardly lead him to attempt. In nature this experiment is performed by many birds of parasitic habits when they lay their eggs in the nests of other birds. Here the eggs hatch and the young alien finds himself squeezed in the nest amongst nestlings of a different species. He is reared under the optimum conditions for the transfer of para-

sites. Thus these birds, the cuckoos and the cowbirds and some others, are exposed at the very beginning of life to infestations of many diverse sorts.

But what are the results of this experiment of nature? Have the cowbirds, for example, acquired a diverse assortment of lice? In order to answer this question a special study has been made of the lice of the common cowbird, *Molothrus ater*, this host having the parasitic habit best developed of any of the cowbirds. Dr. Herbert Friedmann (1, p. 189) in speaking of this species in his book, "The Cowbirds," has stated that it lays its eggs in the nests of no less than 158 species of birds. These belong to 8 orders, 25 families and 103 genera. Many scores of skins of this cowbird were carefully searched by the writer for the presence of lice. Of this large number only 13 were found to possess any. Eight of these were infested with a species of *Philopterus* of the type already reported from icterid hosts, being in fact only slightly different, yet specifically distinct, from two species found on our blackbirds. Of the other five skins, two were infested with a species of *Degeeriella* of a type quite characteristic of icterids, being very similar, yet distinct, from our blackbird-infesting species and even more distinct from our species found on the meadow-lark. One was infested with a species of *Myrsidea*, probably *M. bonariensis* Malcomson, found on a South American cowbird. A specimen of *Goniocotes*, evidently a straggler, was taken from another skin. The remaining skin had on it a specimen of *Degeeriella* and a nymph of *Myrsidea*.

Thus it is seen that our common cowbird is only moderately parasitized, and that its lice are those characteristic of the group of birds to which it belongs.² This is certainly a most remarkable condition considering the

² Kellogg (3, pp. 478-480, pl. liv, fig. 6) has described from our cowbird a species, (*Docophorus*) *Philopterus transpositus*, which apparently belongs to a group found only on parrots. I was unable to take this species from the skins examined. It must be one of rare occurrence on cowbirds.

fact that their young do not have the usual contacts with their parents during the nesting period, but with the young, as well as the adults, of many diverse species. This experiment of nature has given the entomologists and parasitologists a crucial test of the host-group specificity of certain types of biting lice. It does not follow, however, that all other groups have such a tenacious attachment for their original hosts.

THE KANGAROO-DOG LOUSE

It has been stated that parasites may occasionally leave their natural hosts and transfer to others of a quite different sort, provided that in so doing they find a similar "ecological environment." An outstanding example of this kind is found in the kangaroo-dog louse, *Heterodoxus longitarsus* (Piaget). This louse not only is a native of Australia and an original parasite of the kangaroo, but all of the members of its subfamily, the Boopinae, belong exclusively to Australian marsupials. Following the introduction of kangaroos into this country for zoological gardens and circuses this louse has spread to dogs and is now found on these domestic hosts in many parts of the warmer regions of the world. And where it occurs on dogs, frequently it is much more abundant than the original biting louse of the dog, *Trichodectes canis* Degeer.

The possession, in this instance, by the dog and the kangaroo of an identical species indicates the contact of these two diverse types of hosts. But in the case of both host species the "distribution" which brought about the contact was effected through the agencies of man. Had it happened in ancient geological times it would have implied an overlapping in the natural range of the dog and of the kangaroo, an implication which would mean the lack at that time of any natural barrier. Thus the absence of any large body of water, or of a mountain chain, or of an extensive desert, may have been indicated as the conditions should demand.

THE CRAB LICE

As an example of implied relationship between hosts of closely related parasites there is here cited for the first time the case of the crab louse, genus *Phthirus*. The crab louse of man, *Phthirus pubis* (Linnaeus), which infests the body of its host, favoring particularly the inguinal region, is a most unusual species in which the first five segments of the abdomen are ankylosed, and the abdominal segments are produced laterally into large tubercles. Because of these profound morphological changes it has been placed in a family (Phthiridae) by itself.

The writer a few years ago was most surprised to find a second species of crab louse, which infests the gorilla. This species, *Phthirus gorillae* Ewing, is known only from the egg and nymphal instar. Until adults are taken the full significance of its relationship to the crab louse of man remains in doubt. Yet it is most surprising to learn that these two congeneric species—one from man, the other from the gorilla—should constitute a family distinct and apart from all the other sucking lice. To the writer this is one more fact indicating not only the natural relationship of man to the gorilla but also the great antiquity of both man and the great apes. If the crab lice have evolved upon the great apes and man, as apparently they have done, then this evolutionary period must have been long enough to develop family characters in these louse species.

OUR COMMON CHIGGER AND ITS HOSTS

For years the writer has been conducting surveys in different sections of the eastern part of the United States so as to establish the host distribution of the common chigger, *Trombicula irritans* (Riley). This is the mite that in its larval stage is so very annoying to man in certain sections of the country during the hot summer months. As a result of this survey, which has included

the examination of many scores of species and hundreds of individuals of land vertebrates, it is believed that a fair picture of the host distribution of this mite has been obtained. Briefly summarized it is as follows:

The common chigger occurs in nature on certain vertebrate groups, in certain or all stages of the life history of the hosts. It parasitizes four of the five classes of vertebrates—Amphibia, Reptilia, Aves, and Mammalia. Yet the most remarkable thing about this distribution is not the occurrence of the parasite upon so many unrelated hosts but its absence from so many of the host species in all of these four classes.

Of the amphibians only young toads were found infested. Of the snakes only certain land species were infested, while all water snakes and all venomous snakes were without the mites. Of the turtles only one species, *Terrapene carolina*, the common box-turtle, was found infested, yet this one turtle host species proved to be a very important source of supply for the chiggers in nature. Of the birds several species were found to harbor chiggers, yet the vast majority of the mites were obtained from a few ground-frequenting land birds such as the Carolina wren and the towhee. Of the mammals the rabbit was found to be the only important host, and nearly all other wild species were without chiggers.

In many instances the absence of the chiggers on certain groups of hosts was easily explained because the habits of the hosts did not expose them to attack. Unattached chiggers occur only in ground litter where there is sufficient depth of the same to insure moisture in the bottom layers for most of the year. Chiggers are probably absent from most amphibians, from water snakes, water turtles, water birds, and other water species because these hosts do not frequent the particular places where the mite larvae are. Birds that feed in the air and build nests above the ground, as well as those that nest and feed exclusively in trees, would not come in contact with chiggers. On the other hand certain game

birds, such as the bob-white, that feed on the ground, nest on the ground, and roost on the ground, are found to be heavily parasitized with chiggers. Young toads are infested with chiggers, but the adults are not. The absence of the mites from the adult toads may be explained by the presence of repellent glands. Similarly the absence of chiggers from many, if not the most, of the mammals may be explained. Chiggers are easily repelled, as has been shown repeatedly, by any oil or other liquid with a moderate to strong odor. Persons who are largely immune to chigger attack frequently may owe this immunity to a body odor.

In casting about to find an analogous case to that of our common chigger in its host distribution a somewhat similar one has been found. It is that of our rabbit-bird tick, *Haemaphysalis leporis-palustris* Packard. This tick, which does not attack man or most of the other mammals, is very abundant on rabbits. Most birds are not attacked by it, but many birds that feed, nest or roost on the ground are parasitized. In addition to not being found on man, the rabbit-bird tick also differs in its host relationships from those of the chigger in not being reported on cold-blooded vertebrates. Thus, up to date the host distribution of our common chigger appears to be the most unusual in its "spotted" diversity of that of any ectoparasitic species yet studied.

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SHORTER ARTICLES AND DISCUSSION

RELATION OF CHROMATID CROSSING OVER TO THE UPPER LIMIT OF RECOMBINATION PERCENTAGES¹

THERE is abundant evidence from *Drosophila* and other organisms that as the map distance between genes increases the recombination percentage increases toward 50 as an upper limit. In linkage group I of maize, for example, the map distances and recombination values for certain genes are approximately as follows:

Map distances		Recombination values	
<i>P</i>	0		
<i>as</i>	25	<i>P-as</i>	25
<i>f₁</i>	58	<i>P-f₁</i>	41
<i>an</i>	75	<i>P-an</i>	45
<i>bm₂</i>	127	<i>P-bm₂</i>	49

The differences between map distances and the percentages of recombination have been accounted for by the occurrence of multiple crossovers (Morgan, 1919). Jennings (1923) has shown that crossing over without interference or with interference extending on the average over a distance of not more than 30 map units should give recombination values not exceeding 50 per cent. These conclusions were arrived at on the basis of the then accepted hypothesis that crossing over takes place between undivided chromosomes. It is important, therefore, to point out the obvious, but to the best of our information unpublished, relation between the occurrence of chromatid crossing over and the fact that the percentage of recombination between two linked genes, irrespective of how great their map distance may be, does not in general exceed 50. It is our contention that random chromatid crossing over, rather than multiple cross-overs with restricted interference, is the fundamental mechanism which results in recombination percentages approaching 50 as a limiting value.

¹ Paper No. 198, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

It has been shown in *Drosophila*, *Zea* and *Habrobracon* that crossing over occurs when the chromosomes are split equationally into two strands or chromatids. It has further been shown by the work of Anderson (1925) and Bridges and Anderson (1925) that only two strands exchange parts, or cross over, at any one level. Therefore at each point of crossing over there are two crossover and two non-crossover strands. If in a given bivalent there is always one point of crossing over between two loci it follows that 50 per cent. of the strands will be crossover strands and 50 per cent. will be non-crossover strands and the percentage of recombination between the two loci will be 50. If two points of crossing over occur between the two loci and if, as the *Drosophila* data on attached-X's suggest, the identity of the two strands involved in the second point of crossing over is not influenced by the identity of the two strands involved in the first point of crossing over, the proportion of resulting strands is 1 non-crossover : 2 single crossovers : 1 double crossover. Since the double crossover strands have the two loci in the parental combination the ratio of parental combination strands to recombination strands is 1:1. The following table, which was taken from Belling (1931), shows the relation between the number of crossover points to the frequency with which different types of strands are recovered.

Number of crossover points	Types of recovered strands					
	0	1	2	3	4	5
	Non-cross-over strands	Single crossover strands	Double crossover strands	Triple crossover strands	Quadruple crossover strands	Quintuple crossover strands
1	16	16				
2	8	16	8			
3	4	12	12	4		
4	2	8	12	8	2	
5	1	5	10	10	5	1

As the above table shows, the ratio of parental combination to recombination strands is always 1:1, irrespective of the number of crossover points which occur between the loci of any two genes whose inheritance is being studied. Therefore, the

percentage of recombination between any two genes, which is determined by the ratio of recombination strands to the total number of strands, can never exceed 50. Since Belling's table is merely the expansion of $(a + b)^n$, where "a" stands for non-recombination strands, "b" for recombination strands, and "n" for number of crossover points, it should give the same result as chromosome crossing over without interference, as calculated by Jennings (1923) from the same formula. The important consideration here is that these calculations for results of chromatid crossing over are quite independent of the relative frequency of single, double and other multiple crossovers, i.e., independent of interference. They are true for no interference, for complete interference, and for any degree of interference. Furthermore, they hold whether or not crossing over occurs between sister strands.

These conclusions are true only if crossing over between the different strands at the different crossover points is random,² as the *Drosophila* data on attached X's indicate. If, however, crossing over is not random; if, for example, in a bivalent chromosome in which two crossover points have occurred, chromatids a' and b were involved in the first crossover point and chromatids a and b' were always involved in the second crossover point, there would result four single-crossover strands. With such a selective mechanism it would be possible to have more than 50 per cent. of recombinations. This might, perhaps, explain the data of Clausen (1926) on *Viola* and of Wellensiek (1929) on *Pisum* where more than 50 per cent. of recombination was found.

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² Crossing over can be made non-random by the exclusion of sister-strand crossovers without influencing the results.

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FURTHER EVIDENCE OF LINKAGE IN SIZE INHERITANCE

IN a former communication to this journal, the writer (1931) was able to show an indubitable association in heredity between certain size and color characters in the back-cross generation of a mouse interspecific cross (*Mus musculus* x *Mus bactrianus*). The greater weight, humerus, femur and tibia lengths and body length, characteristic of the recessive *musculus* parent, were found associated with brown coat color derived from the same parent, while greater body and tail lengths likewise tended to be characteristic of those animals manifesting the recessive gene for dilution. In those instances in which the mean difference between dominants and recessives equaled or exceeded four times its probable error, genetic linkage between quantitative and qualitative characters was considered as demonstrated. Since such linkage had not previously been demonstrated in mammals, this interpretation did not meet the unqualified approval of all geneticists (Castle, 1932). Hence, further evidence derived from a study of adult weight in the F_2 generation of the same cross may be of interest.

The use of weight as a measure of the size of an organism as a whole, while not entirely satisfactory because of fluctuations brought about by the amount of food ingested, degree of obesity, etc., has the advantage of being easier to obtain than other criteria of size. In this note, weights of *bactrianus*, *musculus* and F_1 (*musculus* ♀ x *bactrianus* ♂), backcross and F_2 hybrids

are considered. All back-cross mice and nearly all animals of the other generations were weighed on the 181st day of life. The exceptions were weighed from one to three days later. All individuals included were born and raised in our laboratory at Bar Harbor, Maine.

Since a great majority of the F_1 and F_2 animals were females (having been produced primarily for a study of spontaneous tumors arising in old age), the weights of all male mice used in this report have been converted into female equivalents and both sexes treated together. These were obtained by dividing the mean female weight of each generation by the mean male weight and then multiplying the weights of the individual males by the quotient obtained. These quotients varied from .829 for *bactrianus* to .899 for the back-cross mice. Previous studies have revealed no sex-linked factors influencing weight, so the procedure is probably justifiable.

123 individuals of *Mus bactrianus* possessed a mean weight of $13.0 \pm .11$ grams with a range extending from 9.6 to 18.3 grams while 116 *musculus* had a mean of $22.4 \pm .19$ grams, ranging from 15.2 to 30.8. There is consequently a slight degree of overlapping in the two forms. 70 F_1 mice were intermediate in weight, although nearer to the larger parent, with a mean of $20.2 \pm .21$ grams and a range from approximately 13 to 27 grams. The back-cross generation, consisting of 293 individuals, varied in weight from 13 to 31 grams with a mean of $19.5 \pm .12$. 219 F_2 's ranged from 7 to 35 grams with a mean of $18.9 \pm .21$, thus extending beyond the limits of the two parent species.

The coefficients of variation show the following results:

Generation	Coefficient of variation
<i>bactrianus</i>	$13.78 \pm .60\%$
<i>musculus</i>	$13.82 \pm .62\%$
F_1	$12.67 \pm .73\%$
back-cross	$15.58 \pm .44\%$
F_2	$23.82 \pm .81\%$

From these figures it appears that the parent races and the F_1 hybrids differ but little in variability. The back-cross mice, on the contrary, are more variable than the F_1 , possibly significantly so since the difference is 3.4 times its probable error. The

F₂ generation is much more variable, the difference being 10.2 times its probable error.

Since the parent species differ in three pairs of color genes, the back-cross of the F₁ to the triple recessive *musculus* produces approximately equal numbers of dominant and recessive members of each of the factor pairs. If the "marked" *musculus* chromosomes carrying the recessive genes for color also possess genes influencing weight, the back-cross mice showing the recessive characters will tend to be heavier than those with the dominant allelomorphs. If size is not influenced through chromosomal genes, or if no such genes are present on any of the three "marked" chromosomes, the recessive members of the factor pairs will exhibit no tendency to exceed the dominant. All intense mice were compared with all dilute, black with brown, and agouti with non-agouti with the results listed below.

No.	Color	Mean Weight	Difference	Difference	
				Probable error	
149	Intense	19.5 ± .17 grams			
144	Dilute	19.5 ± .17 "	0.0 ± .24 grams		0.0
143	Black	18.6 ± .15 "			
150	Brown	20.3 ± .17 "	1.7 ± .23 "		7.4
155	Agouti	19.3 ± .15 "			
138	Non agouti	19.7 ± .19 "	0.4 ± .24 "		1.7

In the F₂ generation, three times as many dominants as recessives are expected in each of the factor pairs. A comparison of the two classes gives the following figures:

No.	Color	Mean Weight	Difference	Difference	
				Probable error	
168	Intense	19.0 ± .25 grams			
51	Dilute	18.8 ± .33 "	-.2 ± .41 grams		0.5
166	Black	18.3 ± .21 "			
53	Brown	20.7 ± .49 "	2.4 ± .53 "		4.5
165	Agouti	18.5 ± .22 "			
54	Non-agouti	20.2 ± .47 "	1.7 ± .52 "		3.3

From the back-cross and F_2 data it appears obvious that there is no difference in the weights of intense and dilute mice. The browns, however, are heavier than the blacks to an undoubtedly significant degree. In the comparison of agoutis and non-agoutis the situation is less clear. Non-agouti mice are heavier than agouti in both generations but only slightly so in the back-cross, while the difference in the F_2 generation is merely of doubtful significance. The data, however, are confirmatory of the former finding, at least in so far as adult weight is concerned, that genes influencing size are found on the chromosome with the gene for brown coat color.

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A COMPARATIVE ANATOMICAL STUDY OF A MUTANT *AQUILEGIA*

AN anatomical study of the "*compacta*" mutants of the European columbine, *Aquilegia vulgaris*, has yielded indirect evidence as to the possible action of the responsible gene. A more complete analysis is under way; in the meantime the pertinent facts are summarized below.

DESCRIPTION

The homozygous recessive *compactas* differ from normal garden columbines (*A. vulgaris*) in a number of ways. (1) They are shorter, bushier and much more branched. In Fig. 1 are shown diagrammatic drawings, made to scale from actual specimens. (2) The stems are more brittle than those of the normal plant, so much so that in walking about in the experimental garden one has to be careful not to brush them roughly, a precaution quite unnecessary with other *Aquilegias*. (3) The most striking peculiarity of *compacta* is the position of the flower. In normal *A. vulgaris* the bud droops and not until the petals

are about to fall does the flower become erect. In the *compacta* mutants the bud is erect even before the first anthers have opened. (4) In families segregating for *compacta* and non-



FIG. 1. Diagrammatic drawings (to scale) of the main shoots of normal (B) and mutant (A) *Aquilegias*. Circles represent flowers and triangles represent leaves.

compacta it is possible to illustrate a fourth difference, a slight effect on the proportions of the petals and the sepals. The laminae of the petals are shorter in the mutants and the sepals, on the average, are less well developed.

ORIGIN

Nothing is known as to the ultimate origin of the mutation other than the fact that it is commonly found among cultivated *Aquilegias*. A dwarf white variety, homozygous for *compacta*, is offered by a number of seed houses under the name of "*alba nana compacta*". In its general features the mutant resembles the "*compacta*" or "*nana compacta*" freaks, known for a large number of cultivated plants.

INHERITANCE

Compacta behaves as a simple Mendelian, single-factor recessive. The material with which we are working was originally given us by Miss Brenhilda Schafer, of the John Innes Horticultural Institution (Anderson and Schafer, 1931), who has graciously permitted us to refer to her unpublished results.

ANATOMY

An anatomical study was undertaken to determine the structural basis for the difference in height and rigidity between the normals and the *compactas*. Sections of the stem were cut from the following parts of the plants: (1) The base of the central shoot (cross and longitudinal sections); (2) the peduncle of the

bud, taken at a point 2 cm. below the base of the bud (cross-sections); (3) the peduncle of the fruit, taken at a point 2 cm. below the base of the fruit (cross-sections).

For both normal and dwarf plants two genetic types were used; one having blue flowers and one with white flowers.

The anatomical structure of the mature *Aquilegia* stem as seen in cross-section is briefly as follows: A large soft pith is surrounded by a ring of bicollateral vascular bundles, each with a cap of schlerenchyma over its outer surface. Encircling the ring of bundles is a sheath of schlerenchyma, which in turn is clothed with a narrow layer of thin-walled parenchyma and an epidermis.

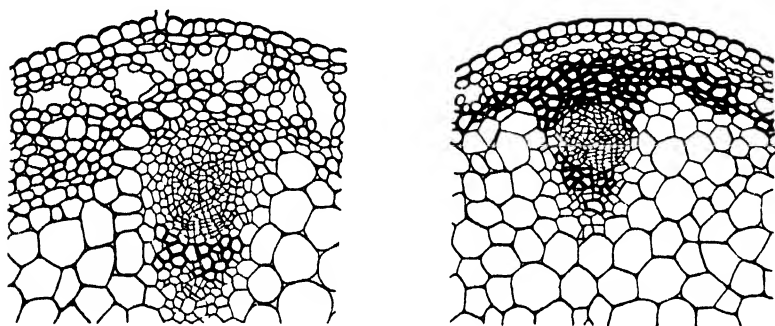


FIG. 2. Cross-sections of the peduncles of young buds of normal (left) and mutant (right) *Aquilegias*.

The most noticeable difference between cross-sections of normal and dwarf stems was obvious under a hand lens. In the base of the stem from the normal plant, the thick-walled, small-lumened cells capping the bundles are often separated from those of the outer layer by intervening large-lumened cells, while in the same part of the dwarf plant the bundle caps are continuous with the outer schlerenchymatous sheath. The secondary walls of the cortex schlerenchyma are thicker in the dwarf than in the normal type. The metaxylem elements appear to have slightly thicker secondary walls in the dwarf plants.

The contrast between normal and dwarf plants is greater in the younger stems (Fig. 2) than in the mature tissues (Fig. 3). Stiffening of the stem seems to occur at an earlier stage in the *compactas* than in the normals. Cross-sections of the peduncle at equal distances below the buds of normal and *compacta* plants showed the basis for this difference. At a distance of 2 cm from

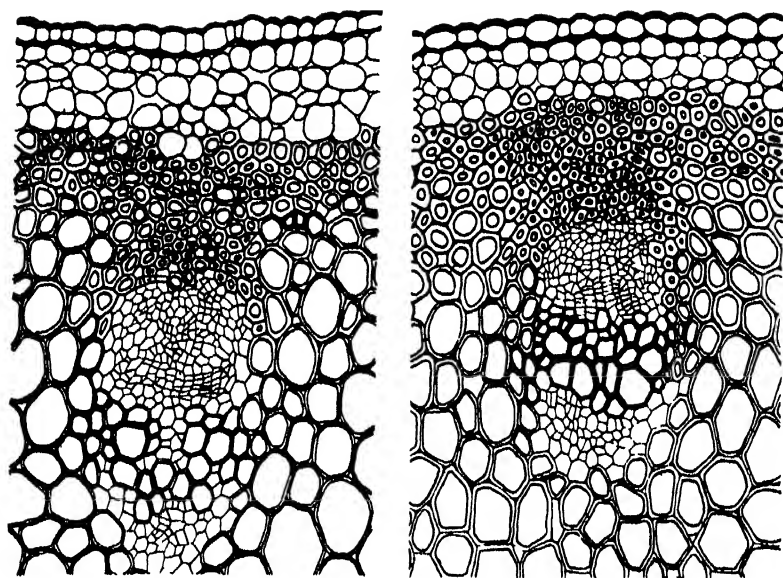


FIG. 3. Cross-sections of the main shoots of normal (left) and mutant (right) *Aquilegias*.

bundle, but in the normal type the sclerenchymatous ring in the cortex is made up of moderately thick-walled but large-lumened cells, while in the dwarf type the ring is made up of cells similar in cross-section to those of the cap.

CONCLUSION

The evidence so far obtained suggests that the manifold effects of the *compacta* gene are all directly or indirectly the result of precocious secondary thickening of the cell walls. Since thickening begins earlier in the *compactas* the flower is upright at a much earlier stage. Since it begins before elongation is complete, it results directly in dwarfism and indirectly in a bushy, prostrate habit of growth. When the main stem can elongate no more, the side branches are pushed out and these in turn start precociously, giving rise to vigorous tertiary branches. Side growth continues in this way, resulting in a plant form

similar in origin and in appearance to a hedge plant which has been repeatedly sheared by the gardener. The greater brittleness of stem in the mutant is probably another result of the greater secondary thickening which, beginning at an earlier stage, reaches a more extreme condition.

An increasing number of Mendelian mutants have been shown to support Goldschmidt's (1927) generalization that genes affect times and rates of development. Particularly interesting by way of contrast to our case is the study by Jenkins and Gerhardt (1931) of the "lazy" gene in maize. There the mutant plants differ from the normals in that secondary thickening of the cell wall takes place at an abnormally *late* stage in stem development.

The anatomical evidence presented above suggests that the *compacta* mutants differ from the normals only in the fact that secondary thickening of the cell walls begins at an earlier stage in stem development. Secondary thickening is *apparently* continued as long in the mutants as in the normals. The secondary wall is *apparently* of the same constitution in the mutants as in the normals. Secondary thickening begins so much earlier in the mutants that the difference is *apparently* one of time of development rather than rate of development. Critical evidence on these three points is still lacking. Provisionally, we may conclude that the *compacta* gene causes its peculiar effects through the precocious initiation of a normal feature of normal development.

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PROGRESSIVE CHANGE IN THE INSECT POPULATION OF FORESTS SINCE THE EARLY TERTIARY¹

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ONE afternoon some twenty years ago during a winter sojourn in northern Florida the writer was collecting insects in a pine forest given over to the production of turpentine. Following the usual practice, the bark and sapwood of each tree had been cut away for a distance on one side and a pot hung at the bottom to catch the resin. The scarred trunks of the disfigured trees were reeking with oozing resin, and here and there insects of various kinds were imbedded where they had been trapped by the sticky exudation. Mrs. Brues was with me at the time and, knowing that I was interested in amber insects, she suggested that we make a collection of these insects from the turpentine trees to compare with the fossil fauna of the amber. We could see several interesting possibilities in such an undertaking and proceeded forthwith to gather the material. The specimens were transferred to vials of alcohol and after a time we had amassed a considerable and very miscellaneous assortment which assumed a much better appearance after the alcohol had dissolved the envelope of resin.

The matter had to be held in abeyance for many years, until I had been able to devote some time to a study of one small part of the insect life of amber. It then appeared that the Florida collection was too small to be of

Side from the Entomological Laboratory, Harvard University.

any considerable value, and another more extensive one was then made in what appears to be a much more suitable region. This I shall deal with in a few moments.

Amber is fossilized and greatly hardened resin. It occurs in lumps or nodules of various sizes, practically in the same form that it first developed as an exudation of sticky resin on the trunks of pines in the amber forests. These pieces are often of quite considerable size, frequently weighing several pounds, and represent the only known remains of magnificent forests that flourished in northern Europe in upper Eocene times. As the fresh resin exuded, its sticky surface trapped innumerable small flying and crawling insects, together with other objects that fell or were blown against it, to be engulfed and permanently preserved just as we now mount small insects in Canada balsam. Many fragments of the plants that grew in the amber forests are thus preserved also, enabling us to gain a far clearer idea of the insects and associated plants than is possible in the case of any other extinct insect-fauna.

As to the flora of the amber forests we are able to form quite a complete picture from the bits of plant remains that are preserved in amber nodules. It is believed that the most abundant tree was a true pine, *Pinus succinifera* Göppert, from which the amber was very probably derived, at least in great part. However, this tree was associated with an extensive and varied sylvan flora which included other conifers of the living genera *Sequoia*, *Taxodium*, *Picea*, *Thuja* and a somewhat dubious form known as *Pinites*. Among deciduous trees there were abundant oaks of numerous species, as well as chestnuts, beeches, bays (*Myrica*), magnolias, cinnamons (*Cinnamomum*) and several palms. The composition of the arborescent flora is therefore not exactly reproduced anywhere at the present time, but is quite similar to that of North America. In the eastern United States most of these trees have living relatives, several types restricted to the south, but the most abundant ones now more com-

mon farther north. Some have pointed out the great similarity of the present flora of Florida, particularly on account of the palmetto palm, magnolia and bay-tree, but the agreement here seems no greater than in New England, on account of the spruces and beeches, and certainly the terrain of the amber forests, as we know it from the caddis-fly fauna, was not that of the flat coastal plain. Undoubtedly the climate was much milder than the one which we now enjoy in the northeastern states, and the forests were clearly comparable with our own, as they existed several centuries ago before their exploitation was begun by civilized man.

The history of Baltic amber after its deposition on the tree-trunks has been quite accurately traced. The amber forests occupied an extensive area in Europe north of the Baltic sea. The amber remained after the death and disintegration of the trees which had produced it, and some is now found in a deposit of so-called "blue earth" which represents the amber-bearing strata of upper Eocene age. Here the amber was apparently carried by streams or rivers and thus accumulated. Much amber is found also in certain stratified sands of Miocene age, where it has been redeposited, commonly in rich pockets. Still later, smaller amounts were distributed by inland ice at the time of glaciation in the north. Most of the original deposits now lie below the surface of the Baltic Sea, where they can not be worked systematically, but from time to time through the agency of severe storms pieces are dislodged. As the amber is lighter than water it rises and drifts ashore where it may be recovered. On occasions as much as a ton or more has been collected on the shores of the Baltic after a stormy night. For a study of the inclusions the amber is commonly sawed into rectangular blocks, each containing an individual specimen and the surface polished to permit observation through the transparent matrix. Much of the amber is not transparent, and as the included insects, trash and other objects are scattered at random, the discovery and prepara-

tion of good specimens is a very slow and laborious process. Thus prepared, examination under the microscope is readily possible, even under high powers, except where flaws in the amber, clouding of the inclusions or superposition of parts interfere with clear vision. Ordinarily the more minute specimens are better preserved than the larger ones, as there is less clouding from water vapor or mould, and they are less apt to have become broken during the process of submergence in the resin.

Thus nature has preserved a very considerable fragment of the rich and varied forest fauna that flourished in Northern Europe some 40,000,000 years ago.

For a number of years I have been interested in a small part of this insect fauna of Baltic amber, comprising a series of families of parasitic species belonging to the order Hymenoptera. After some time spent in sorting, classifying and describing the new genera and species found in the amber I gradually became aware of certain apparent differences in the representation and diversity of the several groups in the fossil amber fauna and that which survives it at the present day. At first blush these seemed to indicate progressive changes in the numerical abundance of certain groups of insects that are well represented in both the amber and recent fauna.

Such changes are certainly to be expected, for we have abundant evidence in practically all groups of animals and plants that evolutionary change has entailed the appearance of numerous particular groups that have waxed abundant and later waned, often to extinction. Due to the limitations of the case, however, the paleontologist must ordinarily concern himself primarily with the morphological aspect of such changes as they relate to the appearance of types, families or genera and their diversification into lesser types and species. Aside from comparative anatomical studies, speculative deductions and the application of certain principles, our entire knowledge of the phylogeny of animals and plants rests upon this

very firm foundation of observed factual evidence. One aspect of the composition of fossil faunas lends itself to observation in only a very crude and incomplete way, since we can not estimate with any degree of accuracy the proportionate numerical abundance or dominance of particular groups or of individual species. This is, of course, due to the fact that certain happy combinations of circumstances are requisite for the preservation of organisms as fossils. Moreover, we know that such combinations have by no means necessarily been similar on different occasions. Most deposits that contain insect remains have been laid down in water, but we are unable to do more than surmise what portions of the prevalent insect fauna have been preserved in any particular bed. We can piece together little evidence concerning the terrain or the flora of the immediate vicinity, nor are we able to say what part transport by wind or carriage by streams may have played in assembling the population sample that we may be fortunate enough to unearth after the lapse of long periods. If certain groups or species are well represented by specimens we know that they must have formed a considerable part of the insect population, but if others are less numerous it is quite probable that they may have for some reason escaped entombment and even if some are absent we may easily find a good reason for their failure to chance upon that spot at the appropriate time. The great preponderance of winged ants in the fauna of the Miocene shales of Florissant is very probably due to storms of volcanic dust which overwhelmed them in flight, while the contrasting abundance of wingless worker ants in the Baltic amber is no doubt due to the more peaceful method by which they were trapped. Thus neither deposit can afford data for an accurate census of the sexual and worker phases of ants during tertiary times.

To return again to the collection of amber insects mentioned previously, it will be seen that this affords an opportunity to compare with considerable accuracy the

Oligocene with the recent insect fauna. We know that the amber fossils are species that frequented the trunks of pine trees in areas of mixed forest quite similar to those now present in the northeastern United States. Furthermore, the genera of insects known from the amber show that this Eocene fauna was more similar to that of the nearctic region at the present time than to the living fauna of any other single part of the world. From his studies on the Trichoptera or caddis-flies of the amber, Ulmer (1912) has reached conclusions concerning the topography of the amber forests which supplement and confirm those drawn from a knowledge of the types of trees present there. The trees indicate a partly mountainous country, and the caddis-flies seem to demonstrate without question the existence of rushing mountain streams, slowly flowing brooks and quiet ponds. Since many genera of caddis-flies are closely restricted in habitat, Ulmer concludes from the representation of modern genera in amber that the number of genera and species requiring mountain streams greatly exceeded the other types, although the latter were present in sufficient abundance to show clearly the existence of the static or quiet water habitat.

These considerations indicate that ecological conditions in the amber forests are rather closely reproduced in the hilly forested areas of New England at the present time, and as already indicated the flora and insect fauna were quite similar to those now existing in that region.

It seemed, therefore, that this region offered a particularly good opportunity to compare in detail the numerical abundance of specific groups of insects as they are preserved in amber with the present forest fauna. This required that a census of forest insects be taken under conditions closely approximating those which led to the formation of the amber inclusions.

In order to collect a closely similar sample of the insect population we made use of the well-known tanglefoot fly paper, which is easily obtainable and proved to be

eminently suited to the purpose. Tanglefoot simulates closely in its sticky surface fresh exuding resin; the sheets may be readily tacked to the trunks of trees and later removed for inspection, so that they serve to collect just that portion of the forest fauna directly comparable with the one found in the amber. It may be asked what differences may be expected when the piney odor of fresh resin is replaced by that of the tanglefoot. There is every reason to believe that odor has little influence in trapping the insects, since most of the species in amber are very evidently in no way directly associated with resin or with the trees which produced it. The trapping of most specimens is undoubtedly by pure accident, *e.g.*, the various Diptera which represent over 50 per cent. of the whole population both in the amber and tanglefoot collections. Even in the case of bark beetles of the family Scolytidae, where some of the amber species must have been associated with the resin-producing trees, the representation of this family is extremely low and quite similar to the scarcity noted in the tanglefoot collection. I believe therefore that no considerable differences in sampling have been introduced by the use of tanglefoot in place of resin.

The localities selected for the tanglefoot collections were all in the township of Petersham, which lies in northern Massachusetts, at an elevation of from 800 to 1,100 feet above sea-level. As nearly as possible areas of well-matured (including some nearly primeval) forest were chosen where there was either a predominance of conifers (white pine and hemlock) or a mixed growth including also considerable beech, oak, maple and birch. The terrain included areas of well-drained higher land, damper spots, open spaces and the borders of both swiftly and slowly flowing brooks. Thus, as nearly as could be judged, the localities which scattered for some miles through the Harvard Forest and on land adjoining our own summer home, represented the several ecological conditions known to have existed in the amber forests and in about the same proportion.

Collections were made in the several localities during the course of the entire summer of 1930 from early May till late September. Sheets of the tanglefoot fly paper were fastened by tacks at each corner to the trunks of trees at different heights that could be conveniently reached from the ground. After a few days numerous insects were caught and the surface of the tanglefoot became less sticky. The papers were then removed, each carefully curled into a cylinder with the sticky surface inside, caught thus with pins and brought into the laboratory. The immersion of each cylinder in a large jar of 95 per cent. alcohol for a few minutes dissolves the matrix and allows the specimens to float free. In spite of this treatment the insects are in very good condition and after removal to vials of clean alcohol may later be conveniently sorted in watch glasses under the microscope. During the course of our work both Mrs. Brues and myself commented frequently and vehemently on the great abundance of blackflies, mosquitoes and biting flies (*Chrysops*) in the forest, and gloated in prospect over the swarms of these pests that would be engulfed in the tanglefoot before the end of the season. Subsequently, when the material was sorted we were pained to learn that only 26 mosquitoes, three *Chrysops* and 18 *Simulium* appeared in the collection of 22,938 specimens, more than 21,000 of which were insects. This is an extreme case, but shows very clearly that the wielding of a net or some other method of collecting in the forest would have produced a very differently selected sample of the insect population, which could not have served for adequate comparison with the amber.

The census of the tanglefoot material has occupied some time and would have been delayed still longer had it not been for very considerable aid from Mrs. Brues and my secretary, Mrs. O'Connor, who did much of the tedious preliminary sorting. As may be seen from the accompanying tabular list, the specimens belonging to some of the more abundant orders have been sorted to

families; other groups, such as the Corrodentia and Trichoptera, have not been thus divided, as my knowledge of the families was not sufficient to insure accuracy in placing broken or otherwise defective specimens.

CENSUS OF TANGLEFOOT POPULATION

Collembola	60	Psychodidae	165
Orthoptera		Chironomidae	652
Stenopelmatidae	11	Culicidae	26
Acrididae	1	Simuliidae	18
Thysanoptera	6	Bibionidae	3
Corrodentia	209	Mycetophiloidae	2,648
Mallophaga	1	Cecidomyiidae	141
Homoptera		Rhagionidae	906
Membracidae	2	Tabanidae	3
Cereopidae	106	Therevidae	1
Jassidae	1,685	Asilidae	4
Cicadellidae	472	Empididae	559
Aphididae	81	Dolichopodidae	3,070
Chermidae	11	Phoridae	6,443
Hemiptera		Platypezidae	1
Tingitidae	18	Pipunculidae	21
Nabidae	3	Syrphidae	99
Miridae	51	Muscoidea	
Odonata	1	Thecostomata	287
(Zygopteran nymph)		Haplostomata	1,195
Plecoptera	35	Coleoptera ²	
Plecoptera	124	Carabidae	8
(incl. 2 nymphal cases)		Silphidae	14
Neuroptera		Scydmaenidae	12
Hemerobiidae	7	Staphylinidae	31
(incl. 2 larvae)		Pselaphidae	4
Coniopterygidae	5	Scaphidiidae	2
Mecoptera		Cucujidae	1
Meropidae	2	Lycidae	3
Panorpidae	37	Lampyridae	43
Trichoptera	332	Cantharidae	87
Lepidoptera		Melyridae	1
Noctuidae	4	Cleridae	1
Geometridae	6	Mordellidae	101
"Micros"	153	Meloidae	1
Nymphalidae	1	Pyrochroidae	1
Caterpillars	12	Oedemeridae	1
Diptera		Elateridae	52
Tipuloidea	260	Eucnemidae	1

² I am greatly indebted to Dr. P. J. Darlington, of the Museum of Comparative Zoology, who checked the series of Coleoptera and examined all doubtful specimens in this order.

Throscidae	74	Ichneumonidae	492
Dryopidae	1	Chalcidoidea	46
Dasillidae	7	Mymaridae	13
Helodidae	25	Roproniidae	1
Dermestidae	6	Serphidae	4
Ptinidae	1	Belytidae	152
Cryptophagidae	1	Diapriidae	23
Erotylidae	1	Scelionidae	25
Mycetaeidae	1	Calliograptidae	57
Colydiidae	11	Platygasteridae	114
Lathridiidae	27	Dryinidae	3
Endomychidae	2	Cynipoidea	8
Coccinellidae	2	Formicidae	67
Alleculidae	4	(including one larva)	
Melandryidae	26	Vespidae	5
Anobiidae	12	Other wasps	10
Cerambycidae	3		
Chrysomelidae	5	OTHER ARTHROPODS	
Curculionidae	8	Diplopoda	9
Scolytidae	3	Chilopoda	1
Scarabaeidae	1	Araneida	1,045
Hymenoptera		Chelonethida	3
Xyelidae	1	Acarina	13
Pamphiliidae	1	Phalangida	179
Tenthredinoidea	8		
Braconidae	59	Total	22,938
Alysiidae	76		

More than half, in fact 71.9 per cent. of the entire tanglefoot collection, are Diptera, 16,484 in all; next in abundance are Homoptera with 2,429, followed by Hymenoptera (1,165) and spiders (1,045). All other orders, together with scattering other arthropods, make up less than 8 per cent. of the collection. This must not, of course, be regarded as a sample of the entire forest population, but it is, as previously indicated, a sample on which we may rely to determine with at least some measure of accuracy what changes have taken place in the forest population during the long period that has passed since the amber was laid down.

For this purpose it will be most satisfactory to examine first two of the groups whose representation in the amber is especially well known. For one of these we have accurate and extensive data furnished by Klebs (1911). As a pioneer student of amber inclusions, Dr. Klebs

amassed an enormous collection, later bequeathed to the Geologisch-paläontologisches Institut at Königsberg. The comparative representation of the more abundant families of Coleoptera in the population of Baltic amber and in the present-day New England fauna is shown in the accompanying table (Table I). Great divergence

TABLE I
COMPARATIVE REPRESENTATION OF THE MORE ABUNDANT FAMILIES OF
COLEOPTERA IN THE POPULATION OF BALTIC AMBER AND THE
PRESENT-DAY NEW ENGLAND FAUNA

	Amber	Per cent.	Recent*	Per cent.	Recent, Actual Numbers
Carabidae	72	3.7	27	1.4	8
Silphidae	9	0.4	47	2.4	14
Scydmaenidae	28	1.4	40	2.0	12
Staphylinidae	69	3.5	103	5.3	31
Pselaphidae	24	1.2	13	0.7	4
Cucujidae	17	0.9	3	0.2	1
Lampyridae	3	0.1	143	7.5	43
Cantharididae	51	2.6	290	14.3	87
Mordellidae	115	5.9	337	17.3	101
Hylophilidae	53	2.7	0	0.0	0
Anthicidae	49	2.5	0	0.0	0
Elateridae	286	14.1	173	8.9	52
Eucnemidae	48	2.4	3	0.2	1
Throscidae	17	0.9	247	12.2	74
Helodidae	376	19.3	83	4.2	25
Dermestidae	5	0.3	20	1.0	6
Cryptophagidae	16	0.8	3	0.2	1
Colydiidae	14	0.7	37	1.9	11
Lathridiidae	41	2.1	90	4.8	27
Alleculidae	17	0.9	13	0.7	4
Melandryidae	44	2.3	87	4.7	26
Anobiidae	236	12.1	40	2.0	12
Cerambycidae	39	2.0	10	0.5	3
Chrysomelidae	30	1.5	17	0.9	5
Curculionidae	47	2.4	27	1.4	8
Scolytidae	37	1.9	10	0.5	3
Totals	1780	91.2	1863	95.5	585

* These numbers have been increased, each in proportion, to compare directly the two populations on the basis of equal size, i.e., the figures in this column are each increased by three and one third times.

will be noticed in the abundance of certain families in each fauna and a very marked change from one fauna to the other is shown in the case of practically every family that is especially abundant either in the amber or in the tanglefoot collection. I do not believe, however, that any definite trend is indicated, such as an increasing abundance of highly specialized types. This might appear to be true, for example, with reference to the increased abundance of Mordellidae, but this is at once offset by an increase in several unquestionably primitive families like the Carabidae, Lampyridae and Cantharididae. The last two indicate, however, a much increased abundance of the more highly modified Lampyridae. It seems, however, that several much specialized families have decreased very noticeably. We may therefore gain little information from the beetles, but as I shall show in a moment they serve to supplement evidence supplied by other groups.

On another tabular chart (Table II) I have listed the several families of parasitic Hymenoptera that I myself have studied in the Baltic amber, together with the data gathered from the tanglefoot collection. An examination of these figures shows a series of consistent changes which appear to be highly significant. Among the ichneumon flies, the two dominant families, Ichneumonidae and Braconidae, which are both well represented in each fauna, have changed places. The more primitive Braconidae, which are somewhat more abundant than the Ichneumonidae in amber, have been reduced in number to about one fourth that of the Ichneumonidae. Among the Serphoidea the families Scelionidae and Platygasteridae have likewise changed places. These two families are very similar, but no one can question the more primitive nature of the Scelionidae. The latter have dropped from 15.6 per cent. to 2.4 per cent., while the population of Platygasteridae has increased from 1.5 per cent. to 10.6 per cent. Another family of primitive wasps, the Bethyliidae, does not appear at all in the tanglefoot col-

TABLE II
COMPARATIVE REPRESENTATION OF THE FAMILIES OF PARASITIC HYMENOPTERA
IN THE POPULATION OF THE BALTIC AMBER AND THE
PRESENT-DAY NEW ENGLAND FAUNA

	Amber	Per cent.	Recent*	Per cent.	Recent, Actual Numbers
Aulacidae	4	0.2	0	0.0	0
Stephanidae	5	0.3	0	0.0	0
Evaniidae	35	1.9	0	0.0	0
Megalyridae	28	1.5	0	0.0	0
Pelecinopteridae	3	0.2	0	0.0	0
Braconidae					
(incl. Alysiidae)	331	18.2	227	12.4	135
Ichneumonidae	292	16.1	832	45.8	492
Chalcidoidea					
(excl. Mymaridae)	191	10.5	78	4.3	46
Mymaridae	26	1.5	22	1.2	13
Roproniidae	0	0	2	0.1	1
Serphidae	74	4.0	7	0.4	4
Diapriidae					
(incl. Belytidae)	258	14.2	296	16.2	175
Scelionidae	285	15.6	43	2.4	25
Platygastridae	27	1.5	193	10.6	114
Calliceratidae	46	2.5	97	5.3	57
Bethylidae	175	9.6	0	0.0	0
Embolemitidae	6	0.3	0	0.0	0
Dryinidae	12	0.7	5	0.3	3
Cynipoidea	5	0.3	13	0.7	8
Mutillidae	11	0.6	0	0.0	0
Chrysididae	3	0.2	0	0.0	0
Totals	1817		1817		1073

* These numbers have been increased, each in proportion, to compare directly the two populations on the basis of equal size, i.e., the figures in this column are each increased by 69.5 per cent. from those in the following column as accurately as whole numbers will follow.

lection although it was represented to the extent of 9.6 per cent. by a series of highly specialized types in the amber. At the present time this family is unquestionably more abundant in warmer regions, which might account to some extent for a lesser abundance in the tanglefoot, although certainly not for its total absence, since it occurs very generally though sparingly in our region. As

this family is one which appears to represent a degenerate type related to the primitive wasps, this diminution in numbers is to be expected. One family, the Megalynidae, represented by a rather common extinct genus in the amber, occurs now only in Australia and South Africa and illustrates a condition noted among other groups of insects that certain types now surviving only in Australia or Malaya existed in the amber forests. Without going into further detail it is clear that the census of these parasitic Hymenoptera shows a quite consistently increased numerical abundance of phyloneanic types with a concurrent decrease in phylogerontic ones in the tanglefoot collection. Before attempting to evaluate these findings, I should like to call attention to another difference in the composition of the two faunas. If we examine the census figures with reference to the variety of types that make up the main bulk of the population³ in the two faunas (Table III), we find that about one half the population of the tanglefoot belongs to a single family, the Ichneumonidae; two families make up almost two thirds; three make up three quarters and four make up seven eighths. In the amber population the number of families making up these several parts are 3, 4, 5 and 7. Thus more different types are well represented in the amber sample. Also, if we count all the families of parasitic Hymenoptera represented, we find

TABLE III
PARASITIC HYMENOPTERA

	Amber	Recent
50 per cent. of population	3 families	1 family
66 per cent. of population	4 families	2 families
75 per cent. of population	5 families	3 families
87 per cent. of population	7 families	4 families
Entire population	20 families	12 families

³ In this way we may avoid a possible cause of error due to the certain rare types which may easily be absent in one sample and present in another.

twenty families in the amber (9 of these absent in tanglefoot) and only twelve in the tanglefoot (1 of these absent in the amber). The last data are not so convincing as the first, since the tanglefoot collection was smaller and several families might have been added if it had been as extensive as the amber one.

I can see only one conclusion to be drawn from these facts relating to the parasitic Hymenoptera. This group was more diversified in the amber forests and several primitive types were represented by more numerous individuals, while several derived ones were numerically less abundant than now. From the restriction in the number of larger groups we must conclude that this series was in a more active state of evolution in the upper Eocene than it is now, that certain gerontic types are being eliminated and that the population includes fewer dominant types. In other words, this group of insects is decadent from an evolutionary standpoint. We can not attempt to say whether the behavior of these insects has become more diverse, more highly adaptive or more complex, as we have only morphological data. However, the persistence of many amber genera to the present day combined with the considerable number of previously unknown structural types (including a new family) found in the amber make it seem very probable that a knowledge of the habits of these insects in the upper Eocene would show that their behavior was certainly as varied and complex as it is at the present day.

Among the Hymenoptera the ants furnish an extreme case of divergence between the amber and tanglefoot. The amber ants have been very carefully studied by Wheeler (1914), who examined 7,819 specimens from the collections of the University of Königsberg. This same collection yielded the 1817 specimens of parasitic Hymenoptera already mentioned. On this basis we should expect to find 467 specimens of ants in the tanglefoot collection if the ants had retained the same numerical ratio to the parasitic families. Instead, there are

only 52 ants, or about 11 per cent. of the expected number, indicating a great decrease in the ant population since the time of the amber, at least in this region. As ants certainly appear to be much more numerous in many tropical countries it appears that the numerical decadence of ants has not extended to the tropics. Professor Wheeler tells me that he believes a similar tanglefoot census in Australia, where ants are particularly numerous, might reveal a population as large as that of the amber forests. This in itself may be regarded as supporting the conclusion that ants are becoming less numerous since the Australian biota is on the whole the most archaic one now in existence.

If we tabulate the Coleoptera as we did the parasitic Hymenoptera, from the standpoint of population groups we find that there was a greater variety of types in the amber fauna (Table IV). Three families made up one

TABLE IV
COLEOPTERA

	Amber	Recent
50 per cent. of population	3 families	4 families
66 per cent. of population	9 families	6 families
75 per cent. of population	12 families	8 families
87 per cent. of population	22 families	12 families
Entire population	62 families	39 families

half of the population; nine made up two thirds; twelve made up three fourths and twenty-two made up seven eighths. For the tanglefoot the corresponding numbers are 4, 6, 8, 12. The amber data are for a larger collection than the tanglefoot, but it will be noticed that our table shows 62 families in the amber and only 39 in the tanglefoot. We have good reason to believe that diversification occurred much earlier among Coleoptera than among the other orders of holometabolous insects, at least with reference to the forest fauna⁴ which occupies a very stable environment. If we are here concerned with an older

⁴ Cf. Brues, '27.

group we must suppose that it is now further past its prime than the Hymenoptera, which I believe to be the case, especially as it formed only 2.5 per cent. of the whole insect population of the tanglefoot compared with about 4 per cent. in the amber.

It may now be asked how far we can extend these considerations to other groups of insects. This question may be approached first by a comparison of the numerical abundance of members of several other orders in the amber and tanglefoot. Unfortunately, I am unable to deal with the matter completely, as there is no reliable census of the amber fauna for several important groups. Those which may be considered are included in Table V.

TABLE V

	Amber	Amber	Mean	Recent	Trend
	Per cent.	Per cent.	Per cent.	Per cent.	
Thysanura	0.1	0.1	0.1	0.0	—
Collembola	6.4	10.6	8.5	0.2	—
Trichoptera	4.6	5.6	5.1	1.5	—
Lepidoptera	0.1	0.1	0.1	0.7	+
Hemiptera	3.1	7.1	5.1	10.8	+
Diptera	56.9	50.9	53.9	71.9	+
Coleoptera	3.6	4.5	4.0	2.5	—
Hymenoptera	3.4	5.1	4.2	5.0	±
Araneida	3.7	4.5	4.1	4.5	±
Acarina	6.7	8.6	7.6	0.05	—

The representation of these orders in the amber is given in percentages of the whole, based on two sources, and the average is in the third column. As these data do not distinguish between the several neuropteroid and orthopteroid orders, a number have necessarily been omitted. The data for the tanglefoot collection are in the fourth column and finally at the right has been added the general trend as to either increase or decrease in abundance. Four large groups, Thysanura, Collembola, Trichoptera and Coleoptera are clearly less numerous in individuals and three others, Lepidoptera, Hemiptera and Diptera are conspicuously more abundant. The Collem-

bola are a very primitive type. So are the Hemiptera, but it will be noticed in the tanglefoot collections that the great abundance of this order is due to a very large number of a single type, the leaf-hoppers. Contrasting Trichoptera with Lepidoptera, the latter are the more primitive and have decreased. Diptera show an enormous increase in spite of their great abundance already in the amber fauna. Clearly the Diptera are developing rapidly and we must look forward to them as the type that is well on its way to dominate the insect world. These statements refer entirely, of course, to population or abundance of individuals and although speciation or the number of specific types in the several groups frequently shows parallel deviations, such a correlation does not necessarily exist.

As to the proportionate abundance of the families of Diptera in amber we have no numerical statistics but many years ago the most astute Dipterist of his time, Hermann Loew (1861) examined a large series of amber Diptera. He states that among the Nematocera the Mycetophilidae (*s. lat.*) are the most numerous both in species and in individuals, while the Culicidae are the most poorly represented. This agrees with the tanglefoot series so far as it extends. Among Brachycera, Loew found that the Dolichopodidae far exceed all other families both in species and individuals. Next to this come the Empididae as far as species, but the number of individuals is far less. Here the tanglefoot figures are quite different. Although numerous, Dolichopodidae (3,070 specimens) are second to the Phoridae (6,443) which exceed any other family and form nearly one third of the whole tanglefoot collection of insects.

Phoridae are well represented in the amber, but are far less numerous than they are in the tanglefoot collection. The great abundance of this family is associated with the most diverse assortment of habits and behavioristic modifications, and of secondary morphological adaptations to be found in any group of Diptera. Yet these flies form a compact group with very distinctive charac-

ters of a highly modified and constant type. This suggests that the family, although well fixed as a group, is now in a very active stage of evolution. This conclusion is borne out by their very great present abundance compared with the amber.

Still another series of Diptera are exceedingly interesting in this connection. I refer to the larger muscoid flies, Muscoidea Thecostomata or Calyptratae, acclaimed by all workers as the most recent, most difficult and unstable of all Diptera. These Diptera are surpassed numerically by the smaller Muscoid flies, Muscoidea, Haplostomata or Acalyptratae, evidently a derivative of the other type. They exhibit enormous diversity in structure and appear to represent the group of Diptera destined to become most numerous in the future. Concerning the changes in abundance of these two groups since the amber we have no exact numerical data, but Loew states that the calyptrates were present although rare and that the acalyptrates were very poorly represented by only a small part of the recognized families. Contrasting this with the tanglefoot collection we find a great difference; 287 calyptrates and 1195 acalyptrates, comprising together 9 per cent. of all the Diptera obtained.

Numerous other groups of insects might be considered. True Neuroptera seem to have become less numerous, although not abundant in either fauna. Thysanoptera have clearly decreased; nearly 70 specimens have been studied by Priesner from the Königsberg and Fritsch collections, while only six appear in the tanglefoot, which is proportionately about half as many in the total population.

From the data presented we may conclude that within the rather narrow limits we have chosen, the insect population has manifested considerable change during its long transformation from the depths of the early tertiary amber forest to a modern one which has so far escaped destruction by the axe. There are many possibilities for error to creep into our methods of collecting the mate-

rials and of evaluating the resulting evidence, but the direction of change is clearly consistent among a number of different groups. We find that in the whole population certain components have increased, others have decreased and others have remained of about equal size. Parallel to the differentiation of genera and species there has been a correlated population increase in the more modern types of insects while the reverse has been true in groups that we may regard as primitive or decadent. The demonstration of an actual numerical increase in individuals in some groups and a decrease in others is the important fact which we may surmise but can not examine statistically in the case of other fossil insect faunas.

After this comparison of an Eocene and recent forest insect fauna can we still be sure that this is the age of insects? Are insects still on the increase in numbers and variety, or have they passed the heyday of their existence during tertiary times? We can not compare their numbers as a whole as we have no reliable measure of population density. We have seen, however, that certain groups have changed greatly in their ratio to the entire population, sometimes changing places with closely similar groups. Quite generally we find more specialized types replacing ones from which they seem undoubtedly to have been derived, although both appear commonly in each fauna. Meanwhile a few species here and there appear to have persisted throughout the entire period during which numerous genera and some families have disappeared completely, indicating a great fixity that promises little adaptive change in the future. In some groups that we have been able to compare in detail we find a greater diversity in the Eocene fauna than in the present one.

All these facts lead us to believe that many abundant groups of insects have passed their prime. We may still be in the age of insects, but certainly we do not now witness them coming into bloom.

In closing our discussion we may ask whether this slow process of changing insect populations has any bearing

on the present problems of applied entomology. So far as any influence on the changes which have occurred during the last century, we must undoubtedly answer in the negative. The upsetting of faunal balance through the establishment and spread of insect pests in new regions is a change of such magnitude and rapidity that it completely swamps the effect of any natural adjustments. Here without question the primary stimulus is extrinsic, involving a greatly increased food supply and a great diminution in natural checks to overpopulation. The changes we have considered appear to be wholly intrinsic, dependent upon the organisms themselves. Although they relate only to numerical abundance, they are analogous to and probably correlated with, if not dependent upon, the progressive morphological change characteristic of groups of animals whose descent can be traced accurately over long periods.

At the present time the disintegration of natural faunal areas has so far progressed that changes in insect populations can not be expected to follow any predictable path in the future.

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PREDICTIONS AS TO CHROMOSOME CONFIGURATION, AS EVIDENCE FOR SEGMENTAL INTERCHANGE IN *OENOTHERA*¹

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INASMUCH as *Oenothera* still remains the outstanding example of chromosome cohesion, it is of particular interest to the student of *Oenothera* to determine the causes behind this phenomenon. Some are fully convinced that segmental interchange is at the basis of chromosome cohesion, but others are skeptical. This paper is an attempt to present additional facts indicating the correctness of the segmental interchange theory as applied to *Oenothera*.

Translocations of a non-reciprocal type have been known in *Drosophila* since the work of Bridges in 1919. The idea of reciprocal translocations, or segmental interchange, was brought forward by Belling (1927) to account for the formation of a circle of 4 chromosomes in a hybrid between a standard line of *Datura* and a so-called cryptic type. At that time, Belling suggested that segmental interchange might be responsible for circle formation in *Oenothera* as well as *Datura*. This suggestion was followed up by Håkansson (1928) and particularly by Darlington (1929), who applied the theory to *Oenothera* in considerable detail.

A method of testing the segmental interchange theory was devised independently by Emerson and Sturtevant (1931) and by Cleland and Blakeslee (1930, 1931), and preliminary tests have already been published. It is the

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purpose of this paper to report further and rather extensive tests of the theory.

Before reporting upon the results of these tests, however, a word may be in order regarding the segmental interchange theory as it has been applied to *Oenothera*, and the method by which it may be tested. The theory assumes in the first place that the chromosomes of each so-called Renner complex have a definite and characteristic arrangement of end-segments, which may differ from those of other complexes. The two complexes composing a single form may, therefore, have different arrangements. The old idea that each chromosome of a given genom has a corresponding chromosome in the opposing genom is assumed to apply only in the case of paired chromosomes in *Oenothera*, and even here it is possible that homologies may be confined only to the ends. In the case of chromosomes found within circles, the assumption is that none of these chromosomes are strictly homologous with any other chromosome in the plant, but each chromosome is homologous, with respect to its end segments, to the 2 other chromosomes to which it is united in the circle (see Emerson 1931, Darlington 1929).

In the second place, it is assumed that these differences between complexes, as regards arrangement of homologous parts, have come about as the result of a series of segmental interchanges between non-homologous chromosomes, which have occurred at rare intervals during the evolutionary development of these complexes from a common source. As a matter of fact, segmental interchange is the only conceivable cause for differences in distribution of homologous parts, unless the position be taken that the various complexes now in existence, with their different arrangements, have had separate origins, and are devoid of evolutionary relationship.

The test devised by Emerson and Sturtevant and by Cleland and Blakeslee is really a method of proving that different arrangements of ends actually do exist in the various complexes. Since, however, such differences in

arrangement can only be explained on the basis of segmental interchange, the test may be taken as applying with equal force to the process of segmental interchange itself.

The method by which this test is applied is as follows: (1) A race with only paired chromosomes is selected as a standard line (*hookeri* deV. has been chosen by both sets of authors as the standard). (2) Various complexes are combined with the standard and with each other. From the chromosome configurations obtained in the resultant F_1 hybrids, it is possible to work out little by little the end arrangement in each complex. (3) In certain cases, this process can be aided by recourse to genetical data. (4) As each new complex is brought into the picture, it becomes increasingly possible to *predict* what configurations it will give in combination with certain complexes, as its behavior becomes known with certain others. (5) Such predictions will be fulfilled only provided the complexes involved have constant arrangements of ends, different complexes usually differing in respect to their arrangements. If these predictions are fulfilled, it proves that the assumption upon which they have been made is correct—namely, that end segments are differently distributed in different complexes and each complex has its own specific arrangement—facts explicable only on the basis of segmental interchange.

To date, 9 such predictions have been tested (Cleveland + Blakeslee, '30, 1 pred.; Emerson + Sturtevant, '31, 2 preds.; Cleveland, '32, 6 preds.). Every one of these predictions has been fulfilled, *i.e.*, each complex-combination has been shown to have the configuration predicted for it. No failures have been recorded. Such a record would seem to furnish strong evidence for the presence of different segmental arrangements in different complexes, and hence for segmental interchange.

During the past winter I have made a large number of additional predictions and verifications, and I propose to review these briefly at this time. A word may be

necessary, however, as to the mode of presentation. In order that the reader may see how the predictions were made, and how verified, it will be necessary to consider the data in the same order in which they were accumulated. There is admittedly no logic in the order in which data were obtained. Material was examined microscopically in the order in which it came to hand, plants blooming early in the season yielding on the whole the first fixations, the first embeddings and the first microscopic mounts. But my excuse in following this order is to show how the predictions were made and tested. It will be noticed that tests were made in every case subsequent to the making of predictions.

(1) The first hybrids examined were (*grandiflora* deV. \times *franciscana* deV.) *acuta* and *truncata*. It had been predicted by Cleland (1932) that the *acuta* would have 2 \odot s 4, 3 pairs, and the *truncata* \odot 14. These configurations were found to be actually present.

(2) The next forms examined were hybrids involving 3 California forms, known tentatively as *T + G*, *Devil's Gate* (Dev. G.) and *Dalton* (Dalt.). These quite distinct races will be described elsewhere. They each possess entirely paired chromosomes, so that all normal germ cells produced by them, either egg or sperm, will carry identical chromosome complexes from the standpoint of segmental arrangement. The first hybrids of these to be examined were (*grandiflora* \times *T + G*) *acuta* and *truncata*, and (*hookeri* \times *T + G*). The first had \odot 4 and 5 pairs, the second \odot 14, the third \odot 4 and 5 pairs. The next hybrid to be examined was *Dev. G.* \times *T + G*. It proved to have 7 pairs. This means that the complexes A *Dev. G.* and $^AT + G$ have the same arrangement of ends, and consequently that hybrids of *Dev. G.* will have the same chromosome configurations as corresponding hybrids of *T + G*. Consequently it was possible to predict that A *Dev. G. acuens* would have \odot 4 and 5 pairs, A *Dev. G. truncans* \odot 14 and A *Dev. G. hook.* \odot 4 and 5 pairs. All

3 of these were tested later in the season, and were found to have the predicted configurations.

The next hybrid studied was *Dalt.* $\times T + G$. This also possessed 7 pairs. *^hDalt.* therefore has the same arrangement of ends as *^hT + G* and *^hDev. G.* One could therefore predict that *^hDalt. acuens* would have $\odot 4$ and 5 pairs, *^hDalt. truncans* $\odot 14$, *^hDalt. ^hhookeri* $\odot 4$ and 5 pairs, and *^hDalt. ^hDev. G.* 7 pairs. All 4 were later tested and found to have the required configurations.

(3) *Suaveolens* $\times T + G$ was then examined. Two complex-combinations were present, as expected, namely, *flavens. ^hT + G* and *albicans. ^hT + G*.

Flavens. ^hT + G proved to have 2 \odot s 4 and 3 pairs. From this fact, it was evident that *flavens. ^hDev. G.* and *flavens. ^hDalt.* and their reciprocals must have 2 \odot s 4 and 5 pairs. This was later found to be the configuration of these combinations.

Albicans. ^hT + G was found to have $\odot 12$ and 1 pair, from which it was predicted that *albicans. ^hDev. G.* and *albicans. ^hDalt.* would also show $\odot 12$ and 1 pair, which they did.

(4) Next in order of investigation after *suaveolens* $\times T + G$ was (*chicagoensis* $\times T + G$) *excellens. ^hT + G*. This hybrid turned out to have 7 pairs. From this it was possible to make 14 definitive predictions, as follows: *excellens. ^hDev. G.* and *excellens. ^hDalt.* should have 7 pairs, which proved to be the case. Furthermore, since *^hT + G*, *^hDev. G.* and *^hDalt.* have the same arrangement of segments as *excellens*, they should give hybrids whose configurations are identical with those found in corresponding hybrids of *excellens*. *Excellens* is known in combination with *gaudens*, *punctulans*, *rubens*, *velans*, *flavens* and *^hhookeri*. At the time that *excellens. ^hT + G* was found to have 7 pairs, the configurations of hybrids of *^hT + G*, *^hDev. G.* and *^hDalt.* with *gaudens*, *punctulans*, *rubens* and *velans* were unknown. Since that time, however, 7 of the 12 hybrids whose configurations were thus

predicted have been tested. In every case, they have given the predicted configuration. These are:

gaudens. ^h T + G	⊙ 10
velans “	⊙ 6
gaudens. ^h Dev. G.	⊙ 10
velans “	⊙ 6
gaudens. ^h Dalt.	⊙ 10
velans “	⊙ 6
punctulans. ^h Dalt. (metacline)	⊙ 12

Of a total of 14 predictions, therefore, made from the fact that *excellens* gives 7 pairs with ^hT + G, 9 have been tested, and all have shown the expected configuration.

(5) The next hybrid that was examined was *muri-cata* × T + G. Two complex-combinations were found, namely *curvans*.^hT + G (metacline) and *rigens*.^hT + G.

Curvans.^hT + G was found to have ⊙ 4, ⊙ 8 and 1 pair. It was possible from this fact to predict that *excellens*.*curvans*, ^hDev. G.*curvans* and ^hDalt.*curvans* would also have a ⊙ 4, ⊙ 8 and 1 pair. Of these three, only one has been tested, namely, *curvans*.^hDalt. This was found to have the required configuration.

Rigens.^hT + G was predicted to have a circle of 8 and 3 pairs, on the basis of data available before the form was studied microscopically. The reasoning upon which this prediction was made is as follows:

Let ^h hookeri	= 1 · 2	3 · 4	5 · 6	7 · 8	9 · 10	11 · 12	13 · 14
“ flavens	= 1 · 4	3 · 2	5 · 6	7 · 8	9 · 10	11 · 12	13 · 14
“ velans	= 1 · 2	3 · 4	5 · 8	7 · 6	9 · 10	11 · 12	13 · 14

^hT × G gives ⊙ 4 with ^hhookeri, 2 ⊙s 4 with *flavens* and ⊙ 6 with *velans*. It therefore has in common with ^hhookeri 2 chromosomes which are not present in *flavens*. The only two ^hhookeri chromosomes which are not found in *flavens* are 1 · 2 3 · 4. ^hT + G must have these, therefore, and having them, will give ⊙ 4 as part of its configuration with *flavens*. In order to give a ⊙ 4 with ^hhookeri; an additional ⊙ 4 with *flavens* and ⊙ 6 with *velans*, ^hT + G must differ from ^hhookeri and *flavens* by a single interchange between one, but not both of the

chromosomes which are involved in the circle in *hookeri*, *velans*, and one of the last three chromosomes. Defining 7·8 and 9·10 as the *hookeri* chromosomes involved in the circle with ${}^hT + G$,²

Let ${}^hT + G = 1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6 \quad 7 \cdot 10 \quad 9 \cdot 8 \quad 11 \cdot 12 \quad 13 \cdot 14$

Rigens gives $\odot 6$ with *hookeri*, and $\odot 4$, $\odot 6$ with *flavens*. It therefore has 2 more chromosomes in common with *hookeri* than with *flavens*. These must be the 2 *hookeri* chromosomes which *flavens* does not have. *Rigens* must therefore have 1·2 3·4. Again, *rigens* gives $\odot 8$ with *velans*, and $\odot 6$ with *hookeri*. It therefore has one more chromosome in common with *hookeri* than it has with *velans*. The only chromosomes which *hookeri* has which are not common to *velans* are 5·6 and 7·8. *Rigens* has therefore either 5·6 or 7·8 (not both).

(a) If *rigens* has 5·6, it can not have 7·8, so can not have both of the *hookeri* chromosomes which are involved in the ${}^hT + G$ circle. It can, however, have (1) one, or (2) neither of these chromosomes, as in the following hypothetical formulae.³

rigens (1) 1·2 3·4 5·6 7·12 9·10 11·14 13·8 (would give $\odot 8$
with ${}^hT + G$)

rigens (2) 1·2 3·4 5·6 7·10 9·12 11·8 13·14 (would give $\odot 4$
with ${}^hT + G$)

(b) If *rigens* has 7·8, it already has one of the chromosomes in the ${}^hT + G$ circle, and can have the other or not. If it has both in common with *hookeri*, its formula may be arranged as follows:⁴

rigens (3) 1·2 3·4 5·12 7·8 9·10 11·14 13·6 (would give $\odot 4$,
 $\odot 6$ with ${}^hT + G$)

If it has but one of these two chromosomes in common with *hookeri* (7·8), the formula may be written:⁵

² The subsequent reasoning will apply no matter what choice is made within the limits stated. In this, as in the later choices made in the present line of reasoning, no claim is made that the choice in each case will turn out to be the correct one.

³ Subsequent reasoning will apply, no matter how the ends of the *rigens* chromosomes which enter into the circle of 6 in *rigens hookeri* are chosen.

rigens (4) 1·2 3·4 5·10 7·8 9·12 11·6 13·14 (would give $\odot 8$
with $^hT + G$)

There are then 3 tentative possibilities for *rigens*. $^hT + G$, namely, $\odot 4$; $\odot 4, \odot 6$; and $\odot 8$. These possibilities may be tested in part by the use of *acuens*. *Acuens* gives $\odot 4$ with $^hT + G$ and $\odot 4, \odot 6$ with *velans*. It therefore has only 3 chromosomes in common with $^hT + G$ which are not present in *velans*. There are only 3 $^hT + G$ chromosomes, however, which are not found in *velans*, namely 5·6 7·10 9·8. *Acuens* must therefore have these. But *acuens* gives $\odot 4, \odot 8$ with *rigens*, having but one chromosome in common with the latter. This at once eliminates *rigens* formula (2), since this has 2 of the 3 chromosomes which *acuens* must have. *Rigens*. $^hT + G$ can not have, therefore, $\odot 4$ and 5 pairs. Hypothetical *acuens* formulae can be written, however, which will fit the other *rigens* formulae, as well as all other formulae known, as follows:

acuens = 1·4 3·2 5·6 7·10 9·8 11·12 13·14 (This will fit *rigens*
(1) and (4))
" = 1·2 3·14 5·6 7·10 9·8 11·12 13·4 (This will fit *rigens*
(3))

By the use of *stringens*⁴ it is possible to eliminate one of the tentative *acuens* and hence one of the remaining possibilities for *rigens*. *Stringens* has 1·4 and 3·2 (Cleland and Blakeslee 1931), and gives 2 \odot s 4 with *acuens*. It is possible to construct a *stringens* which will give the correct configuration with *hookeri*, *flavens* and *velans*, and also with the first formula of *acuens*, as follows:

stringens = 1·4 3·2 5·12 7·8 9·10 11·6 13·14

It is impossible, however, to formulate a *stringens* which will give only circles of 4 with the second formula for *acuens*, for the chromosomes 1·4 and 3·2 of *stringens*

⁴At the time this prediction was made, it was not known that *acuens*. *flavens* has $\odot 4$ and 5 pairs. With that information available, the second *acuens* formula would have been automatically eliminated, as giving the wrong configuration with *flavens*; and *stringens* would not have been needed in the argument.

would perforce belong to a circle of larger size if brought into combination with a complex in which one of the first two chromosomes has exchanged with a chromosome further along in the formula, as in the second formula for *acuens*. Consequently, the second formula for *acuens* is impossible. This means, however, that the third formula for *rigens* is also impossible. Only 2 formulae remain for *rigens*, therefore, and both give a circle of 8 with ${}^hT + G$. It was possible, on the basis of this line of reasoning, therefore, to predict that *rigens*. ${}^hT + G$ would have a circle of 8. This would also mean that *rigens*. ${}^hDev. G.$ and *rigens*. ${}^hDalt.$ should have a circle of 8. All 3 forms were tested microscopically, and were found to have the required configurations.

(6) The last hybrids to be examined were *Dev. G* \times *franciscana* deV. and *T + G* \times *franciscana* deV. Since hfranciscana deV. has the same end arrangement as hookeri , it follows that it must give the same configuration with ${}^hDev. G.$ and ${}^hT + G$ that hookeri gives, namely, $\odot 4$ and 5 pairs. Upon examination, these hybrids were found to have this chromosome arrangement.

This completes the list of predictions made and tested during the past winter. They make a total of 28 predictions, every one of which was found to be correct. These added to the 9 predictions previously published make a grand total of 37 predictions which have been tested, every one of which has been proved correct.⁵

Of what significance, then, is this accomplishment? It seems to me that it takes segmental interchange definitely out of the realm of theory and places it in the realm of proved fact. For in the first place, these results indicate that each complex has its own specific and characteristic arrangement of end segments, that different complexes may have different segmental arrangements, and that the

⁵ It should be possible, more and more, as individual genes are assigned to particular segments, to predict genetical linkages in given complex-combinations, as well as chromosome configurations. A beginning along this line has been made by Emerson and Sturtevant (1931).

union of homologous ends will give a definite, characteristic and predictable arrangement of chromosomes in each particular complex-combination. Second, the only possible way to account for the different arrangements of ends in different complexes is, as we have seen, on the basis of mutual exchanges between non-homologous segments—in other words, segmental interchange. In the third place, definite predictions such as these are impossible on any other grounds than those here assumed. It would seem therefore that the data at hand furnish a definite proof of the validity of the segmental interchange theory as applied to *Oenothera*.

I wish to call attention, in conclusion, to the effect of this work upon the problem of genetical linkage in *Oenothera*. The field has been contested by 2 opposing theories. The first, the so-called chromosome cohesion theory (Cleland 1923, 1924, 1926), has assumed that each individual univalent chromosome occupies a definite position within the circle, with paternally and maternally derived chromosomes alternating; that paternal and maternal chromosomes are separated as a rule to opposite poles, inasmuch as adjacent chromosomes are known to go to opposite poles; that the uniform passage of all paternal chromosomes to one pole and maternal chromosomes to the other pole can mean but one thing genetically—a linkage between genes in non-homologous chromosomes belonging to the circle. The extensive linkage found in *Oenothera* and the formation of the Renner complexes are therefore ascribed to chromosome linkage. The alternative theory denies the definite position of individual chromosomes (Shull 1928), claiming that, while homologous chromosomes are probably adjacent in the circle, they may occupy either the right-hand or the left-hand position indifferently, and consequently independent assortment will take place between different sets of homologues as readily when these are within a single circle as when they belong to different chromosome groups. Chromosome linkage has therefore nothing to

do with genetical linkage, all of which is to be explained in the ordinary and conventional way—as due to inclusion within a single chromosome.

But if the segmental interchange theory be correct, then each particular chromosome *must* occupy a given and specific place with reference to the other chromosomes in the circle, for it *must lie adjacent to those chromosomes which have ends homologous with its own*. If segmental interchange be proved correct, then determinate chromosome position is a proved fact, and the only element of doubt that has been present in the argument for the chromosome cohesion theory is thereby removed. If then the evidence from predictions, as I have outlined it, is accepted as a sufficient proof of segmental interchange in *Oenothera*, it follows automatically that chromosome cohesion is responsible for the linkage of genes in non-homologous chromosomes of a circle. Even if the present verdict in regard to segmental interchange is “not as yet proven,” one must at least admit that the concept of segmental interchange inevitably implies that each chromosome of a circle occupies a determinate position within the circle; and if and when segmental interchange becomes accepted as a fact, it will be necessary to accept along with it the chromosome cohesion theory.

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THE RELATION OF THE INDUCED MUTATION RATE TO DIFFERENT PHYSIOLOGICAL STATES IN DROSOPHILA MELANOGASTER: II. IRRADIATION DURING COMPLETE ANESTHESIA¹

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IN investigation of the manner in which radiation effects are produced, the authors undertook to study the possible influence of various internal factors acting along with radiation. The present paper constitutes the second of a series dealing with radio-physiology.

As has been pointed out in the first paper of this series (in press), and others, there can be little doubt that tissues and cells differ in susceptibility and that the sensitivity of a given cell may change from time to time. The same intensity of radiation produces very dissimilar effects upon different kinds of cells and upon cells in different phases of activity or of their life histories. In 1906, Bergonié and Tribondeau (1)² formulated the principle, known as the first law of radiation therapy, that susceptibility varies directly with reproductive capacity and indirectly with the degree of differentiation. Observations by many investigators indicate the high sensitivity of rapidly growing tissues and of cells undergoing the complicated process of division. (2, 6, 8, 9, 10, 11).

Canti and Donaldson (2), studying the effects of radiation on mitosis *in vitro*, found that even a brief exposure to the gamma-rays of radium prevented cells which were about to divide from beginning the process. Unless, however, irradiation is prolonged or very intense, most cells suffer no visible injury but begin to divide again after a short recovery period, giving no evidence of per-

¹ The expenses of this investigation were supported in part by a grant from the committee on the effects of radiation upon living organisms of the National Research Council.

² Items of the literature cited in the first paper are referred to by number.

manent harm. This return to activity after relatively slight exposure, and the fact that in cells under irradiation some part will respond more quickly than another, form the basis of the entire field of those genetic investigations in which lethal and visible mutations, translocations and gene-rearrangement have been produced experimentally in an attempt to solve the problem of the causes of variability among organisms in nature.

In the early days of the therapeutic application of x-rays and radium, the term "selective action" was employed as representing that the rays produced a greater effect upon some cells than upon others. As irradiation gradually came into use both therapeutically and experimentally, observations pointed more and more to tissue and cell specificity, and it came to be generally accepted that this action is not due to any selective power of the rays but to the susceptibility of the cells. Early in radiation therapy the endothelium of the blood vessels was found to be one of the most sensitive of tissues. Finzi and Horsley (1911) in work on the brain came to the conclusion that the changes observed were due almost entirely to changes in the blood vessels, while the direct action of relatively large doses on the cerebral tissues seemed to be extremely slight. That glandular tissue is affected readily was shown as early as 1909 by Dominici's experiments on the skin. Finzi (1913) found gland tissues susceptible to even slight action of x-rays and radium, the cells disintegrating almost at once and gradually being removed by leucocytes. The high sensitivity of gonadal tissues was also recognized early.

Thus it appeared that the term "selective action" focused too much attention upon the radiation and too little upon the response of the thing irradiated, and the terms "sensitivity," "vulnerability," "susceptibility" were used as substitutes. These have been followed by the term "selective resistance," intending to indicate that cells would be more or less affected by radiation, according to their ability to oppose a large or small resistance to its action. The term "selective absorp-

tion" was suggested, but it is now generally accepted that the coefficient of absorption for all kinds of cells, determined by a progressive comparison with that of different metallic screens, is about the same.

Whether the cell is affected at all or what part of it is altered seems to depend upon the physiological condition at the time of irradiation and on the consequent physical and chemical changes. Very little is known as yet of the chemistry of irradiated material, but there is reason to believe that the cell constituents are ionized and cellular ferments activated, so that reactions are possible which otherwise would not occur at all. In different sections both of the same malignant growth and of normal tissues taken before and after irradiation, the staining reactions point to marked changes in chemical composition. Recent investigations (Love, 1931, Spear, 1932) of the effects of radiation *in vitro* indicate that a limit of action of x-rays and radium exists beyond which no further cells are affected as a result of exposure. Love in 1931 found that the diminution curve of dividing cells reaches a plateau and that in order to obtain further reduction relatively greater doses were necessary. It is conceivable, perhaps, that a limit of effectiveness is reached, because at that point all cells in such a physiological state as would render them susceptible have been affected.

THE INFLUENCE OF COMPLETE ANESTHESIA

In an attempt to study radiation effects under varying physiological conditions, both males and females of *Drosophila* were irradiated during complete anesthetization by di-ethyl ether. Exposure was effected in thin gelatin capsules, enclosed in a small double-walled chamber, the bottom and sides of which were constructed of very thin wood. The chamber was open at the top. Between the walls cotton was kept saturated with ether in sufficient concentration that the flies were "just under" the anesthetic throughout treatment. As a reservoir for the ether, a modified Mariotte bottle was employed which was equipped with a U-tube and capillary

thermometer tubing, the flow of ether being restricted thus to a series of timed drops. The small anesthetization chamber containing the capsules was placed as usual in a larger irradiation chamber, the latter being entirely enclosed except for the passage of small-bore rubber tubing which served to carry the anesthetic fluid. Within the irradiation chamber thermometer tubing was not used in order to avoid secondary radiation from the glass.

ETHERIZED IRRADIATED MALES, ETHERIZED IRRADIATED
FEMALES, AND IRRADIATED NON-ETHERIZED
CONTROLS

Here, as in experiments previously reported, the test of radiation effectiveness was the rate of induced lethal mutation occurring among the descendants of crosses of ClB (sc, v, f, bb) by wild. Wild-type males, newly hatched from cultures at the height of productivity, were exposed under anesthetic in this way to an irradiation of 300 milligram-hours (gamma-radiation only) at a distance of ten centimeters. The alpha and beta-rays were excluded by means of a 0.5 mm platinum filter, which at the same time allows the passage of 78 per cent. of the gamma-radiation. Supplementary filtration through a layer of Columbia paste one centimeter in thickness was used. Following exposure, these males were mated to ClB females. Lethal mutations induced in the treated parent generation are detected in the F₂ generation.

The treated females were first generation bar-eyed individuals of the cross, ClB (sc, v, f, bb) by wild. These females receive one x-chromosome carrying the ClB factor and one x-chromosome bearing the wild-type characters. The sc, v, f, bb-chromosome from the female parent goes to the first generation sons, which in this case are discarded. The first-generation females are heterozygous for the lethal and when mated to the wild-type give ordinarily one half the usual number of sons in the F₂ generation, or a 100:50 ratio. A new lethal mutation induced in the chromosome bearing the wild-type characteristics is inherited by the other half of the

sons, and they also fail to appear. As was indicated in the first paper of this series, a number of preliminary experiments demonstrated the occurrence of such an induced lethal in this x-chromosome in proportion to the dosage applied. Such carrier females were irradiated under anesthetic in exactly the same manner as the males. Here the lethal is detected in the generation immediately following treatment.

Both males and females were compared with controls irradiated under identical conditions of treatment—distance, filtration, dosage, exposure time, etc., being the same.

ANESTHETIZED CONTROLS

Previous to Muller's discovery in 1927 that mutations could be produced experimentally by x-radiation, many different kinds of chemicals had been tried without success. Notwithstanding this fact, a series of control experiments was made in order to be certain whether long-continued anesthetization in the absence of irradiation could bring about lethal mutation. Wild-type males and females of CIB by wild were anesthetized separately for a period of one hour and mated afterward in the usual manner. In 2,450 F_2 cultures of etherized males and 2,500 corresponding cultures from etherized females no lethal mutations occurred.

EXPERIMENTAL FINDINGS

Unlike the results from flies irradiated after starvation, no post-irradiative mortality was observed here in the treated parent generation. The comparisons given in Table I and Fig. 1 show at once the high sterility and lethal mutation percentages occurring among the descendants of flies exposed to gamma-radiation under complete anesthesia. The differences in both series of irradiation, that of treated males and that of treated females, are well above the threshold of significance.

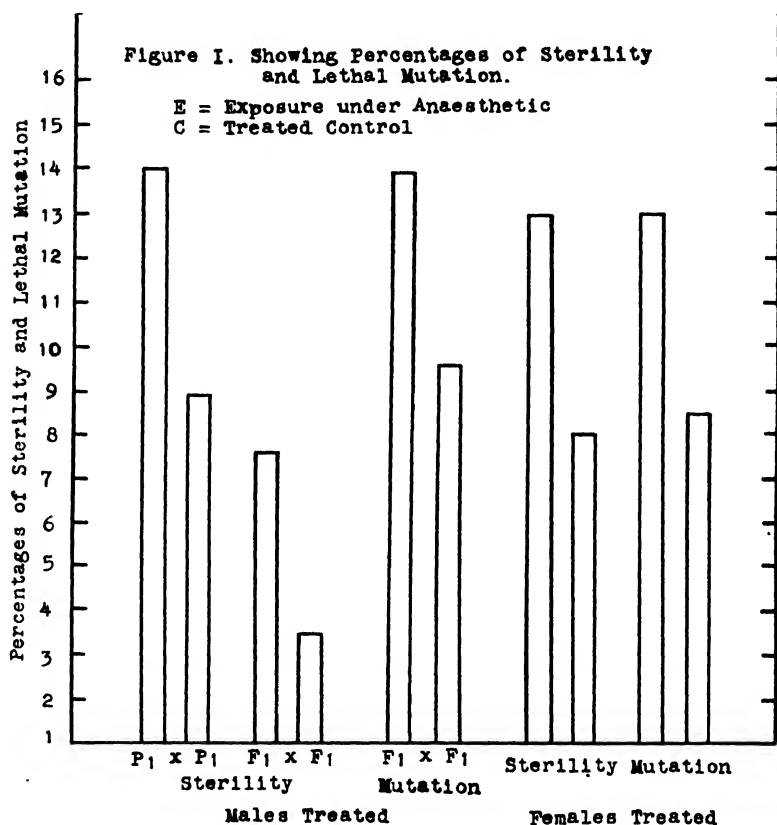
The sterility values are again absolute, representing complete sterility. As in experiments previously re-

TABLE I
SHOWING THE PERCENTAGES OF STERILITY AND LETHAL MUTATION FOLLOWING IRRADIATION OF DROSOPHILA UNDER ANESTHETIC
AS COMPARED WITH TREATED CONTROLS

Generation	Exposure under anesthetic	No. cul- tures	Treated control	No. cul- tures	Difference	Significance
Per cent. of Sterility						
Treated males						
P ₁ × P ₁	14.0067 ± 0.6072	1485	8.9887 ± 1.4451	178	+ 5.0180 ± 1.5620	3.21 × p. e.
F ₁ × F ₁	7.5319 ± 0.3671	2350	3.6923 ± 0.4988	650	+ 3.8396 ± 0.6192	6.21 × p. e.
Per cent. of Lethal Mutation						
F ₁ × F ₁	13.9910 ± 0.4954	2230	9.7444 ± 0.7980	626	+ 4.2466 ± 0.9392	4.52 × p. e.
Per cent. of Sterility						
Treated females	12.9333 ± 0.4833	2250	8.0000 ± 0.4724	1500	+ 4.9333 ± 0.6758	7.29 × p. e.
Per cent. of Lethal Mutation						
	13.0168 ± 0.5060	1959	8.4782 ± 0.5085	1380	+ 4.5386 ± 0.7174	6.33 × p. e.

Total number of initial single-pair matings on which these figures are based: Series of Treated Males—3,835; Series of Treated Females—3,976. Sterility tests are additional to these figures.

ported, all matings giving indication of non-fertility were allowed a period of recovery at the end of which time sterility tests were made. It is interesting to note in the results for treated males, where the lethal is carried by the F_1 female and comes to light in the second generation, a confirmation of earlier findings, *i.e.*, that the F_1 sterility values are approximately one half those for the treated



generation. This consistent reduction in the amount of sterility occurring among individuals two generations removed from exposure suggests perhaps the existence of some factor necessary for fertility which was affected by the treatment. The writers hope to test this point in the near future.

Concerning the slightly different response of the two sexes to identical conditions of irradiation, Table II

reveals that the sterility and mutation values are consistently less when females are treated, although not significantly so. Indications of sex differences have been found by Muller (7, 1929) in a number of experiments with x-rays. The rate of induction in mature sperm was observed to be higher than that in adult females; larval males gave a higher rate of mutation than females rayed at the same larval stage. A greater sex difference in sensitivity was apparent in gene-rearranging effects of the rays than in transmuting effects on individual genes.

The consistent high percentages of sterility and the increased lethal mutation rates obtained in these experiments by irradiation of flies during anesthesia are un-

TABLE II

SHOWING SLIGHT DIFFERENCES IN THE RESULTS OBTAINED WHEN MALES AND FEMALES ARE IRRADIATED UNDER IDENTICAL CONDITIONS OF TREATMENT

Percentage of	Males treated	Females treated	Difference
Treated control			
Sterility	8.9887 \pm 1.4451	8.0000 \pm 0.4724	0.9887 \pm 1.5133
Mutation	9.7444 \pm 0.7980	8.4782 \pm 0.5085	1.2662 \pm 0.9462
Exposure under anesthetic			
Sterility	14.0067 \pm 0.6072	12.9333 \pm 0.4833	1.0734 \pm 0.7760
Mutation	13.9910 \pm 0.4954	13.0168 \pm 0.5060	0.9742 \pm 0.7081

doubtedly significant. It is difficult, however, to say to what extent the differences observed were due to increased sensitivity of the gonadial tissues under anesthetic and to what extent, if any, to mechanical effects. Early in the experimental work it was thought that some secondary radiation might result from the small etherization chamber or its rubber tube attachment. Consequently, controls were irradiated in the chamber with its attachment but in the absence of any anesthetic fluid. The resulting sterility and mutation percentages accord almost exactly with those of the original treated controls, thus eliminating that possibility. Whether increased secondary radiation from the high concentration

of ether vapor within the enclosed treatment chamber might be in part responsible is a question at the present stage of the experiments.

It is conceivable perhaps that ether absorbed by the germ cells may be a sensitizer in combination with radium-radiation and may have a catalytic action for the type of chemical transformation which results in the production of lethal mutation. Changes in protoplasmic viscosity may be brought about by irradiation which might have considerable influence upon the response of the cell to absorbed chemical substances. Haendly (1918), studying changes in irradiated carcinoma cells, observed marked alterations of the degree of hydration and of viscosity, increase or decrease in nuclear size, aggregation or disintegration of chromatin, etc. That sol \rightleftharpoons gel changes occur in the colloidal constituents of nerve cells during anesthesia is generally accepted. Possibly it is not running too far afield to suppose that similar changes in germ cells might sensitize them to radium-irradiation.

The above suggestions are offered merely as an attempt to explain the phenomena observed in our experiments. The results obtained pave the way for similar studies of a more detailed character in analysis of just how the effect is produced. Accordingly, the experiments are being continued with the idea of studying the physical agents employed and the induced physiological conditions of the animals.

SUMMARY

In preliminary studies of the physiology of mutation production, males and females of *Drosophila* were irradiated during complete anesthesia by di-ethyl ether. Unlike the results reported in the first paper of the series where the flies had been irradiated after starvation, both males and females exposed under anesthetic to gamma-radiation of 300 milligram-hours showed no mortality in the treated parent generation, but gave high percentages of sterility and a high rate of lethal mutation as compared with treated controls. In controls exposed to an-

esthetic alone no signs of sterility were observed, and no lethal mutations occurred. There was a slight difference in the response of the two sexes to identical conditions of irradiation, the females being consistently a little less sensitive. These differences are, however, not statistically significant. The experiments are being continued with a view to analyzing just how the effect is produced and to determining to what extent the results obtained are due to increased sensitivity of the gonadial tissues and to what extent, if to any, to mechanical effects.

The authors take pleasure in expressing their appreciation to Drs. A. Lacassagne and G. Gricoureff, of the Radium Institute, and to the Radiological Clinic of the Curie Foundation for arranging the radium exposures.

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GENIC MODIFICATIONS IN *DROSOPHILA* *MELANOGASTER* INDUCED BY HEAT IRRADIATION

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Mutations induced in 5-6 day old larvae of *Drosophila melanogaster* by exposure to a high temperature have been reported by Goldschmidt, Jollos and Rokizky. Ferry, Shapiro and Sidoroff, in their experiments, which were a repetition of Goldschmidt's work, failed to find any mutations. In order to offer additional information relative to the effect of high temperature on the mutability of *D. melanogaster*, the authors repeated Goldschmidt's experiments with slight modifications suggested by Jollos. Further information was sought relative to the response of variously aged larvae, as well as the adults themselves, when exposed to high temperature.

A Florida culture of wild type *D. melanogaster* was closely inbred for three generations before a stock was made up for the heat irradiation experiments. Seventy-two flies were paired, and three pairs were placed in each of 12 vials, measuring 1×4 inches, which had been quarter filled with banana agar. After 24 hours the flies were transferred to fresh vials, and this process was repeated until five sets of 12 vials each were obtained. Variously aged larvae, 1-2, 2-3, 3-4, 4-5 and 5-6 days old, respectively, were then available for experimentation.

Ten vials of each set were treated at 36° C. for 15 hours, the other two vials of each set being reserved for untreated controls. Immediately after the exposure the vials were emptied into half pint bottles, containing a

¹ The authors are grateful to Dr. D. E. Lancefield, of the department of zoology, Columbia University, for helpful suggestions, and to Dr. C. R. Plunkett, of New York University, for a culture of Florida wild type *Drosophila melanogaster*.

corn-meal-syrup-agar culture medium. The immediate addition of fresh food undoubtedly greatly reduced the mortality rate of the larvae, which otherwise would have had to live in over-fermented food. All flies were kept at 26° C. before and after the exposure to 36° C.

As soon as the flies hatched, mass cultures were made, usually placing three or four pairs of flies in a culture bottle, though occasionally as many as five pairs were bred in a single bottle. It is quite possible that the bottles were overcrowded and that some mutants, which are presumably weaker than the wild type, were lost. Very few bottles, however, failed to yield numerous flies, and comparatively few bottles were discarded on account of excess bacterial or mold growths.

Flies were obtained from 13 of the 50 cultures treated in five series of 10 cultures each. In the series in which the larvae were 1-2 days old when treated, four cultures yielded adults; 2-3 days old, one culture; 3-4 days old, none; 4-5 days old, two cultures; and 5-6 days old, six cultures. Disregarding the cultures which failed to yield any adults at all, the average number of flies per vial for 1-2 day old larvae was 7.3; 2-3 day old, 14; 3-4 day old, 0; 4-5 day old, 15.5; and 5-6 day old, 3.7, while the controls averaged 63.6 flies per culture. The mortality rate among the treated flies was relatively high. The total number of treated larvae which hatched into viable adults was 96. In the progeny of these flies a total of 8 distinct mutations were discovered, an additional mutant, sooty, showing in the hatched larvae. Tables I and II present data which show the number of cultures and flies included in the experiment, and the number of the genic modifications obtained, respectively.

The mutations arose in the following numbers: Among the 29 viable 1-2 day old larvae, 6; the 14 viable 2-3 day old larvae, 2; (no 3-4 day old larvae hatched); the 31 viable 4-5 day old larvae, 1; and the 22 viable 5-6 day old larvae, 0. Apparently the larvae which were 1-2 days old when exposed to heat irradiation responded to temperature change to a greater degree than the other

TABLE I

THE AGE OF LARVAE OF *DROSOPHILA MELANOGASTER* TREATED AT 36° C. FOR 15 HOURS, THE NUMBER THAT SURVIVED AND THE NUMBER OF THEIR OBSERVED PROGENY

Age of larvae	Number cultures yielding flies	Number flies hatched	Number flies in F ₁	Number flies in F ₂	Number flies in F ₃
1-2 day	4	29	2,088	7,906	7,092
	*2	118	219	1,444	1,037
2-3 days	1	14	1,041	6,664	4,070
	*2	117	2,621	2,597	1,119
3-4 days	0				
	*2	104	1,420	1,424	2,526
4-5 days	2	31	910	5,289	3,370
	*2	442	628	2,070	1,314
5-6 days	6	22	273	224	531
	*2	130	267	233	631
Totals	13	96	4,312	20,083	15,063
	*10	911	5,155	7,768	6,627

* Untreated control cultures.

groups of variously aged larvae which were exposed to the same treatment.

The same original stock of wild type *D. melanogaster* was used for testing the effects of high temperature on the mutation rate of the adults. Fifty flies were placed in individual glass test-tubes, which were tightly corked and submerged in a constant temperature water bath at the desired temperature for specific lengths of time. Though a total of 8,618 flies were treated, only 1,140 were tested for mutations.

Three additional mutations were found to have occurred among the offspring of these adult flies. The temperatures used and the length of each exposure together with the number of flies treated and the number of their progeny examined are presented in Table III. Of the nine series which were treated, three yielded mutations, sooty, sepia and abnormal legs, respectively.

TABLE III

THE TEMPERATURES, LENGTHS OF EXPOSURES, INDUCED MUTATIONS AND THE COUNTS OF THE PROGENY OF ADULT *DROSOPHILA MELANOGASTER* EXPOSED TO HEAT IRRADIATION

Length of exposure	Temperature °C.	Number flies used	Number flies in F ₁	Number flies in F ₂	Number flies in F ₃	Induced mutations
5 min.	43	41	1,662	1,818	3,872	0
5 min.	42	100	2,350	6,279	6,523	0
10 min.	41	183	5,096	2,309	3,422	1, sooty, F ₂
15 min.	41	46	893	1,287	1,079	0
20 min.	40	195	566	807	683	0
30 min.	40	188	344	1,164	2,232	0
40 min.	39	196	589	747	283	0
60 min.	39	173	7	301	979	1, sepia, F ₂
14 hrs.	36	18	293	1,288	1,304	1, abnormal legs, F ₂
Controls		342	5,032	5,468	6,818	0

A total of twelve distinct mutations was obtained from the heat-treated flies observed in this test, which included a count of over 110,000 flies. Approximately 45,000 untreated flies were examined for mutations, but none were found. Many irregularities were noted, but they were found either to occur among the untreated flies or to be abnormalities that are not inherited. Among these flies eye, bristle, wing and abdomen deformities were found, many of which corresponded to the forms recorded by Goldschmidt, Jollos and Rokizky. In this connection Table IV presents comparative lists of modifications found by Goldschmidt, Jollos, Rokizky and the authors.

The four genic modifications herewith reported, namely, white, sooty, sepia and abnormal legs, were tested through F₂ and F₃ for inheritance and were found to be hereditary genic modifications. The white eye individuals which appeared in three independent cultures were all males. They were crossed to wild type and to

TABLE IV
COMPARATIVE LISTS OF REPORTED GENIC MODIFICATIONS IN *DROSOPHILA*
MELANOGASTER INDUCED BY HEAT IRRADIATION

Goldschmidt	Jollos	Rokizky	Authors
(Florida wild stock)	(Florida wild stock)	(Florida wild stock)	(Florida wild stock)
Aristapedia	Eosin	Rough eye	White
Sooty	Eosin modification factor	Dark body and darker trident pattern	Sooty
Kidney	Black or Sooty		Sepia
Rolled		Notch	Abnormal legs
White	Bobbed	Crinkled wings	
(And many others)	Yellow	Reduced bristle size	
	Abnormal abdomen	Lethal	
	(Spineless stock)	Rudimentary wings	
	Eosin	Deformed wings with raised tip	
	Eosin modification factor	III cross vein	
	Sooty	Indentation of inner edge of wings	
	Black		
	Aristaless		
	Abnormal abdomen		
	(Eosin stock)		
	Black or Sooty		
	Eosin modification factor		
	Abnormal abdomen		
	(Coral stock)		
	White		
	Black		
	Abnormal abdomen		

white stock. The crosses resulted in progeny identical with those obtained when the white stock itself is crossed to wild type or to other white stock. It is a significant fact that the abnormal eye condition found in the wild stock appeared in the white progeny of the white males obtained from heat irradiation when mated to white stock females.

One sooty-like female developed directly from the treated larvae, but it died before offspring were obtained. Goldschmidt reported a similar case, for which there is at

present no suitable explanation. Another sooty female was obtained in the F_2 . It was crossed with wild type and in the succeeding generations behaved as the known stock sooty does, appearing in the heterozygous forms accompanied with a considerably darkened trident pattern on the thorax. The third sooty appeared among the progeny of the treated adults. It likewise showed progeny characteristics of sooty when crossed to wild type.

Sepia was found in three cultures, two in which the larvae were treated and one in which the adults were treated. Of the three females which appeared one was crossed to wild type and the other two to sepia. In the F_2 a ratio of three wild type to one sepia was obtained from the wild type cross, and in the cross with sepia all sepia were obtained in the F_1 and F_2 .

The fourth genic modification, abnormal legs, however, could not be identified definitely with any of the known mutations. It is nevertheless an inheritable character appearing in F_1 , F_2 and F_3 progeny of inbred abnormal legged individuals. In no case, however, was a pure culture obtainable. This character appears in varying degrees of intensity, the least noticeable cases of abnormal legs showing a crescent-like bending and thickening of the basal tarsi of the hind legs, the crescent points turning either towards or away from the body or parallel to the body. In the extreme cases, besides the bending of the tarsi, there is also a bending in the tibiae and in all the five tarsal segments, in all three pairs of legs. Many intermediate forms were observed.

In addition to the above characters found solely in treated flies many hereditary modifications were found to appear in the untreated as well as the treated series. Chief among them was a character restricted to the eye. Such modifications as enlarged eye, indented eye, kidney eye, rough eye, aristaless, reduplicated aristaless, fused facets, papilla-like projections on the eye, bristles completely surrounding the eye, and a partially superim-

posed duplicate eye on either or both eyes, were found in the stock. Numerous irregularities were also found among the dorsocentral and scutellar bristles in both the controls and treated flies. The bristles varied considerably in size and number. Often no bristles appeared on emerging flies, while occasionally as many as five dorsocentral bristles appeared on the newly hatched flies. No mosaics were found among any of the flies examined. Lethals, though probably present, were not identified. Sterility, though stressed by Goldschmidt, appeared definitely in one series only, namely, an exposure of the adults to 39° C. for one hour, when a total of seven flies only were obtained from 3 pairs which were mated individually. All other flies appeared to be normal.

The appearance of twelve mutations among 110,000 flies bred from heat-treated parents, while none appeared among 45,000 untreated flies, indicates that heat treatment greatly increases the rate of mutation. Furthermore, the appearance of half of these mutations (six) among the progeny of heat-treated larvae from 1-2 days old (17,086 flies) apparently indicates that this period in the development of the fly responds to heat treatment at a greater rate than later periods. The mutation rate among the adults was found to be considerably lower, three appearing among 49,317 flies.

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CULTURE MEDIA FOR DROSOPHILA AND THE PH OF MEDIA

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PART I. IMPROVEMENTS IN CULTURE METHODS: THE RÔLE OF YEAST

GAMETIC AND OBSERVED RATIOS

IN the two decades during which *Drosophila melanogaster* has been bred as material for studies on heredity frequent improvements have been made in the culture media and methods in order to secure unhampered development of the full number of eggs laid by a female during a limited sampling period of ten days or less. This is to obtain the closest possible approximation or parallelism between the directly observed phenotypic series or ratios of zygotes on the one hand and the gametic series or ratios which are the real objects of study. The discrepancy is greatest where relatively weak mutant forms must be raised in competition with wild-type or strong forms. In many classes of experiments the accuracy and importance of the results are directly dependent on absence of distortion in the zygotic ratios, for example, in studies where linkage or non-disjunction is involved. Wherever possible the experiments are planned so as to avoid use of mutant types with high mortality. But often no mutant of normal viability is available for some special region of a chromosome, and of course in all experiments to discover the nature and behavior of those mutant types which happen to have low viability the only resource is to employ optimum culture conditions already

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developed and tested. In testing for the optimum methods these mutants of low viability are good indices, for where weak flies do emerge consistently in the closest approximation to the number of wild-type sibs, these conditions are optimum for both kinds.

THE FERMENTED BANANA MEDIUM

In the early work, prior to about 1916, fermented banana was generally used as food for the larvae and adults. The usual technique has been published in the 1915 edition of "The Mechanism of Mendelian Heredity" (page 229). The essentials of the method are the following: bananas with intact skins were put into closed jars, as a protection against the chance of egg deposition by stray flies, and were kept there until they became thoroughly ripe. They were then peeled, and the pulp broken into pieces and dropped into another covered jar containing water seeded with yeast. Usually the yeasty liquid used in the jar was the fermented juice from the previous lot of bananas. Fermentation converted the pieces of banana into a spongy mass floating on top of the liquid. The pulp was at its best about a day after starting the fermentation and became too acid for use after the second day. This rather dry fermented pulp was put into the culture bottles on the bottom, in amounts of about 5 grams for each half-pint bottle and was then covered with a layer or two of paper toweling. The flies were next inserted and the bottle stoppered with cotton. If the flies were inserted while still under ether they were first put into a small paper cornucopia which was stuck to the side of the bottle and in which the flies remained safe while recovering.

This amount of food was found not to be sufficient to give unhampered development to all the larvae that could be produced by a mother laying for the usual period of ten days. The flies tended to be small in size, few in number and highly variable in both respects. Preferably a second supply of food was often added, alongside

the first mass so as not to bury any of the eggs or pupae already present. The new food was added after about 5 days. One of the greatest defects of this system was the fact that the jarring necessary in getting the offspring out was very liable to dislodge the food mass, which would then crush or entangle the flies.

THE ALCOHOLIZED BANANA MEDIUM

In 1915-16 comparative tests were made by Bridges, as the result of which the procedure was improved somewhat (Bridges, 1921). The fermentation was started fresh each time, instead of using the old juice from the last batch of bananas. This delayed the development of acetic acid. The presence of a slight amount of alcohol (optimum 1.5 per cent.) at the beginning of the fermentation was found by test to be conducive to higher outputs. The favorable action of the alcohol is probably exercised in two ways; first, alcohol is a definite stimulant to egg-laying; and second, it is a powerful retarder of the development of competing molds and bacteria. Thus, Baumberger (1919) quotes Lafar (1910, II, p. 238) "From the standpoint of the oecological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon capable of hindering the appearance of other fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation, it also restricts the further development of the producer." In this case, as with yeast poisons in general, the first result is the diminution and later the cessation (at about 6 per cent.) of cell reproduction. A still greater concentration reduces fermentation and brings it to a stop (at 14-21 per cent.), while still more alcohol kills the cells. Recently Richards (1928) has studied the inhibiting effect of alcohol on the growth of yeast and on fermentation and finds it very great. But in the usual bacteria-contaminated yeast cultures used for food for flies the alcohol does not accumulate markedly. Instead, it is changed

over by bacterial action into acetic acid. It has long been thought that the increase in acidity of the culture medium is itself a very important obstacle to the culture of flies. The exhaustion of the sugars and yeast foods, from too little or too poor medium, is perhaps the primary factor in limiting the growing of flies.

In the alcohol method the pulp of ripe sound bananas was weighed and put to ferment in a large, shallow, tightly covered glass dish with an equal weight of 3 per cent. alcohol (optimum percentage). Each banana pulp was crushed slightly, little more than enough to separate it into three longitudinal strips. Yeast was added. The optimum amount of drained pulp, fermented 24 hours, for each half pint bottle was found to be 25 grams—more than twice as much as formerly used. Similarly, the optimum amount of paper was 1.0 gram per bottle, instead of about 0.5 gram as formerly used. This paper was used in the form of strips 5 cm long and 0.7 cm wide, matted down on the food, which was placed directly on the bottom. This method was almost entirely free of trouble from the food mass coming loose. No refeeding was necessary. It was found that pint bottles, with a larger area of bottom, gave larger progenies than half-pint bottles, but the greater convenience in handling the smaller bottles led to their adoption as standard. Another innovation was the use of cheesecloth to cover and reinforce the cotton plugs before insertion. The plugs lasted longer and when removed from the bottle did not, as formerly, leave a fringe of cotton fibers which entangled the flies.

The fermented banana and the alcoholized banana media are still occasionally prepared as an emergency measure, or for raising other species of *Drosophila*, or as a source of food in keeping flies temporarily in vials, or for refeeding old stock or reserve cultures.

THE BANANA AGAR MEDIUM

In the autumn of 1916, Bridges received from R. W. Glaser directions for making the transparent banana

juice and agar culture medium which he had developed in cooperation with Baumberger (Baumberger and Glaser, 1917) for the clear observation of egg-laying and of the growth and behavior of larvae. Tests were made which showed (Bridges, 1921) that the concentration of food in this medium was inadequate for general use, but that the use of agar to hold the medium in place was a decided improvement over matted absorbent paper. Accordingly, the amount and concentration of food was increased by grinding up and using all the banana pulp instead of using only the clear strained juice. The method was modified and developed until in the spring of 1917 it was generally adopted in the laboratory as the standard method. The details of this method have been published (Bridges, 1921; also in the Laboratory Manual by Morgan, Sturtevant, Muller and Bridges; Henry Holt and Company, 1923), and need only brief comment here. The minimum percentage of agar needed to hold the food fairly solid was determined at roughly 0.7 per cent. One per cent. was adopted as standard, since with increased stiffness of medium there was less loss of pupae by being worked into the food and smothered or drowned. For this same reason considerable paper was used also (about 0.5 gram) and pupation would then generally occur on the paper rather than in the food cake. With this method the risk of contamination of cultures through the food supply was greatly lowered, since one step in the preparation involved heating to a temperature more than sufficient to kill any eggs or larvae which might be present in the bananas before they were run through the potato masher and mixed with the agar solution. The concentration of food in the medium was kept high by using only as much water as weighed banana pulp. The amount of food per bottle was kept high by using 50 cc of the medium, equivalent to the 25 grams of banana pulp previously determined as optimum. This method remained standard in the Columbia laboratories for over ten years and is still widely used elsewhere.

THE RÔLE OF YEAST

In 1907 E. Guyenot had published an investigation of the nutrition of flies in relation to microorganisms and concluded that for uniformity of results, whether genetic or physiological, the cultures should be entirely free of living microorganisms. Delcourt and Guyenot finally accomplished this aim (1911). They had first used as culture media potatoes or apples, on which many microorganisms flourished. They then transferred flies to fresh culture tubes containing sterilized potato and selected those lines in which the flies had happened to carry over the smallest variety of microorganisms. They thus secured lines from which they had eliminated one by one all microorganisms except living yeast. For a considerable period they carried on their routine cultures of flies satisfactorily on a medium of potato and the pure culture of living yeast that the flies carried over in the transfers. They were of the opinion that the principal food of the flies was the yeast. Finally they used dead sterilized baker's or brewer's yeast held in a mat by absorbent cotton. The stock of nutrient medium consisted of 300 grams of commercial compressed yeast made up to 1,000 cc with water (30 per cent. yeast). About 50 cc of this medium and sufficient cotton to absorb all free water were placed in Ehrlenmeyer flasks and sterilized. The dead yeast proved excellent for the flies but unfavorable for the living yeast. By repeated transfers they were able to secure strains which were entirely free from living yeast, as well as other microorganisms, and which thrived well on the dead yeast medium.

The work of Delcourt and Guyenot was confirmed by Northrop and Loeb (1917) and by Baumberger (1917a). Northrop and Loeb raised flies many generations with success by essentially the same method as that used by Delcourt and Guyenot, *viz.*, upon dead sterilized yeast held in a mat by absorbent cotton. They rendered their flies free from microorganisms by treatment of the eggs

with HgCl, rather than by repeated transfers. Baumberger secured sterile races much more easily by washing pupae or eggs for 10 minutes in 85 per cent. alcohol.

These researches made it clear that normally the yeast growing on the banana or other media, rather than the banana or fruit itself, was the principal food of the larvae, although other microorganisms and the fruit pulp were undoubtedly utilized as well. Baumberger (1917a, 1919) was of the opinion that a loose symbiosis exists in the fact of the universal transference of yeast by eggs, larvae and the feet of adults. The importance of the yeast as food for the larvae lies principally in its high protein count, approximately 11 per cent., whereas the per cent.) that larvae restricted to sterile banana pulp protein concentration in the banana itself is so low (1.3 require two to six times as long to reach pupation, and then only in greatly reduced numbers and of small size (Baumberger, 1919).

Baumberger stated (1917b) that yeast and flies thrived well on Pasteur's nutrient agar medium, the formula for which he reprinted.

The common name of *Drosophila melanogaster* has been "pomace fly," "vinegar fly" "fruit fly," "banana fly," etc., but in the light of these researches it is seen that it should be called the "yeast fly." Another "fruit fly," *Anastrepha ludens*, the Mexican fruit fly, has been reared by Darby (unpublished) on a nutrient medium with yeast as the food. The question may be asked whether all "fruit flies" may not be supported by a diet of microorganisms.

According to Baumberger (1919) those substances tend to attract adult insects and also to induce egg deposition that are the normal products of the activity of the particular microorganism on which the larvae of the insect principally feed. Not "fruity" odors, but odors characteristic of the activity of microorganisms growing on the fruit are the attractions. Hence alcohol derives its attractiveness to *Drosophila* from the fact that the prin-

cial normal food of *Drosophila* larvae is yeast, the alcohol odor being a sign-post to the presence of yeast.

In the commercial growing of yeast, molasses is used widely, and Baumberger (1917b) reported that in raising flies Pasteur's solution could be approximated by diluting molasses with three parts of water. During 1917 Dr. H. J. Muller used a molasses agar medium at Columbia. His best success came from substitution of Karo corn syrup for about half of the molasses content. One may now guess that the improvement lay in reducing the amount of SO_2 which was present in the bleached molasses. While this method was fairly successful, he abandoned it presently in favor of the banana agar method that had been improved and standardized.

PEARL'S SYNTHETIC MEDIA

In connection with his study on population characteristics, Pearl used *Drosophila* extensively. For uniformity of results he sought to standardize the *Drosophila* culture medium and to this end experimented with synthetic media, following the lead of Delcourt and Guyenot, of Loeb and Northrop and especially of Baumberger. He reported in a preliminary note (Pearl, 1926) and later in detail (Pearl and Penniman, 1926; Pearl, Allen and Penniman, 1926) on these media. The best medium, S-101, had the following formula:

Solution A

Cane sugar	500 grams.
KNa $\text{C}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$	50 "
$(\text{NH}_4)_2 \text{SO}_4$	12 "
Mg $\text{SO}_4 \cdot 7\text{H}_2\text{O}$	3 "
Ca Cl_2	15 "
H ₂ O to make 3000 cc of solution.	

Solution B

Agar	135 grams.
Tartaric acid	30 "
$\text{KH}_2 \text{PO}_4$	6 "
H ₂ O to make 3000 cc of solution.	

Heat the agar in the water to solution, add the salts, and for the medium to be used in the fly bottles, mix

equal parts of solutions A and B. Use 50 cc per culture. Seed with yeast two days before putting in the parental flies.

The authors reported less mortality of parents and much higher output of progeny on medium S-101 than on the banana agar control.

The experience with this medium in the Columbia laboratory was not as happy as hoped for, and the oral reports of other workers did not indicate that they found it much, if at all, more satisfactory than the banana agar method. Greater trouble with molds was reported.

We are indebted to Mr. W. N. Mertz, working at Park College, Missouri, under the direction of Miss Martha Scott, for the data on which Table 1 is based. These data show that for fertile cultures having the same percentage the average productivity on Pearl's medium S-101 was practically identical with the productivity on banana agar. The number of sterile cultures and the variability in productivity was markedly greater on Pearl's medium. The relative viability of the character white eye was about the same on S-101 as on banana agar, but the viability of males was slightly higher, higher even than the number of females. It is unusual for the number of males to exceed the number of females in an experiment, but on account of the high variability shown not much significance is to be attached to this difference.

MISCELLANEOUS MEDIA

The use of starch paste as a substitute for agar or paper as binding material was suggested by Dr. C. W. Metz, who had used this method in 1914 and 1915 for raising species other than *Drosophila melanogaster*. In the spring of 1916 considerable work was done by Bridges in testing out various media containing cornstarch, sugar, peptone and salts, with a seeding of yeast. These methods gave high progenies, but trouble with molds was increased.

TABLE 1
COMPARATIVE PRODUCTIVITIES OF SISTER CULTURES INVOLVING RED AND
WHITE EYES, SOME REARED ON BANANA AGAR, OTHERS ON PEARL'S
S-101 MEDIUM

Banana agar medium							
No.	+ ♀	+ ♂	w ♀	w ♂	Av.	% ♂	% w
24	1821	1354	132	42.6
6	355	351	118	49.7
21	1277	1165	116	47.7	47.7
8	462	451	114	49.4
23	1507	693	741	128	48.8	51.6
11	627	445	319	126	54.9	41.7
23	747	752	733	691	127	49.4	48.7
6	208	230	192	195	137	51.5	46.9
122		15,316			126	48.2	48.1

Pearl's S-101 medium							
No.	+ ♀	+ ♂	w ♀	w ♂	Av.	% ♂	% w
2	129	100	115	43.6
4	221	286	127	56.4
7	382	443	118	53.7	57.7
4	165	185	87	52.8
8	603	381	275	157	52.1	41.9
3	156	119	74	116	55.3	38.3
7	249	274	271	270	152	50.8	51.1
4	91	107	99	101	100	52.3	50.2
39		4,981			128	52.4	48.9

For his work on the distribution of crossing-over in the third chromosome of *Drosophila melanogaster* Gowen (1919) used fermented banana during 1915 and early 1916, but changed in June, 1916, to an artificial food mixture of starch, sugar, peptone, yeast and water. The crossing-over results were unaffected by the change in food material.

Dr. O. L. Mohr, in working in Norway, where bananas were rare, tried various other fruits and was of the opinion in 1920 that dried pear was the most convenient and satisfactory substitute for banana.

Dr. Th. Dobzhansky relates that in Russia he used a raisin agar medium, developed prior to 1923, in the laboratory of Chetverikov. This medium was prepared from 100 parts water, 50 parts raisins, ground very fine by a meat chopper, and 2 to 3 parts agar. This medium has the advantage that molds rarely grow upon it, especially if yeast is added.

Later, on account of the scarcity of agar, the Russian workers have added potato pulp to their media, but at the expense of greater trouble with molds. The formula given by Gershenson (1928) for use with *D. obscura* is: water 800, fermented raisins 100, cooked mashed potatoes 400, agar 4.

Komai (1927) reported on a synthetic medium developed by his coworker, M. Chino. The formula was:

Peeled banana pulp	100 gm.
Kôji	100 gm.
Water	800 gm.

Kôji was described as a culture of *Aspergillus oryzae* on rice, commonly used in Japan for fermenting rice for brewing sake and for other purposes. The kôji supplied protein and was relatively inexpensive in Japan. Komai recommended 34 gm per half-pint culture bottle as compared with the 50 gm of banana agar medium generally used. This medium he found to be cheaper than the banana agar medium, as satisfactory in the rearing of *Drosophila melanogaster* and apparently better for *D. virilis*.

PART II. CORNMEAL MOLASSES AGAR MEDIA

MEDIUM A

After ten years' use the banana agar method became somewhat unsatisfactory from the standpoint of relative cost. The price of bananas was becoming higher and the quantity used was increasing rapidly. The texture of the banana agar medium was also not too satisfactory, in that it was not fibrous or porous, as was the case with

the fermented banana and paper medium. This defect resulted in surface action of the larvae rather than penetration by the larvae through and through the mass. The use of paper helped, but the paper did not mix with the agar thoroughly enough. Bran or oat hulls seemed better, but bran was thought to increase mold and oat hulls were hard to get.

In the spring of 1926, Dr. Helen Redfield tried a number of combinations with molasses as the food and various materials as fillers and stiffeners. All these yielded offspring. They included bran and molasses, agar and molasses, agar and bran and molasses, cooked cornmeal and molasses, cooked cornmeal bran and molasses, cooked cornmeal agar and molasses. It was hoped to develop a formula excluding the expensive agar. The boiled cornmeal medium, without agar, formed a starch paste stiff enough to stand the jarring of the cultures, provided enough cornmeal was used (20 per cent.). But late in the course of the cultures there was a softening of the paste that was troublesome. Also the medium was so viscous that in the process of preparation it was very difficult to put it into the culture bottles. By combination with agar these difficulties were overcome and the percentage of cornmeal reduced. As the source of sugar a mixture of "Brer Rabbit" brand of molasses and Karo corn syrup, as originally developed by Muller, was used. Redfield's formula follows:

For each 100 cc of the medium:

Water	62.5 cc.
Agar	0.8 gm.
Cornmeal ("Quaker Oats")	16.7 gm.
Molasses	11.0 cc.
Karo corn syrup	9.0 cc.

Two thirds of the water was used to dissolve the agar; the molasses was then added. The meal, previously moistened with a third of the water to prevent lumping, was stirred in and the mixture cooked until it stiffened. This food has been used by Redfield with satisfactory results for *Drosophila melanogaster*, *D. obscura*,

D. simulans, *D. hydei*, *D. immigrans* and *D. repleta*. For *D. virilis*, however, it seems not to be as successful as the old banana-agar medium.

For general laboratory use it was thought that the expense of the medium might be further reduced by using a cheap grade of chicken-feed cornmeal. In using this meal in the summer of 1926 Bridges found that the formula should be modified as follows:

For each 100 cc of the medium:

	Water	68 cc.
	Agar	1 gm.
Medium A.	Cornmeal	16 gm.
	Molasses	8 cc.
	Karo corn syrup	7 cc.

In preparing this medium, about a quarter of the water was saved to be added as the last step, in order to thin the stiffened cornmeal to a consistency that could be poured without too much trouble. For pouring, the thinned medium was put into a very large funnel supported by a ring stand. The stem of the funnel was terminated with a large thin-walled rubber tube and the pouring was controlled by a large spring clamp on the tube. About 50 cc was used per half-pint bottle, with toweling paper and yeast on top.

THE pH CHANGES OF MEDIA

Pearl had been of the opinion that a high H-ion concentration (pH 3.0) was favorable to flies because unfavorable to troublesome bacteria and molds. He had investigated the pH changes in the banana agar, and in his medium S-99, rather carefully.

Some work by Darby (1930) had shown that the optimum pH for the growth of yeast is at approximately pH 4.45. The question arose: What pH did the new medium show and could this be modified in a favorable direction.

THE SPOT METHOD OF COLORIMETRIC DETERMINATION OF pH

The technique employed by Darby in making these determinations was essentially the "spot" method of

Felton (1921) with a few modifications for the sake of higher speed in carrying out large numbers of determinations. Since the values obtained at the end regions of the ranges of the various indicators are uncertain, the determinations have been made in duplicate wherever it was possible to check by a second determination with an indicator of overlapping range.

THE pH OF MEDIUM A, WITHOUT FLIES

Ten culture bottles were made up, on April 9, 1927, according to the formula just given, using 50 cc of medium A per bottle. The cultures were seeded with

TABLE 2
MEDIUM A, WITHOUT FLIES; pH READINGS ON SUCCESSIVE DAYS

No.	0	1	2	3	4	5	6	7	8
1	5.7	5.8	6.0	5.2	4.0	3.7	3.7	3.7	3.7
2	5.7	5.7	5.4	5.2	4.4	3.9	3.9	3.8	3.7
3	5.7	5.7	5.4	4.8	3.8	3.7	3.7	3.7	3.7
4	5.7	5.6	5.4	5.2	4.0	3.7	3.7	3.7	3.8
5	5.7	5.7	5.4	4.8	4.0	3.6	3.6	3.6	3.6
6	5.7	5.8	6.0	5.2	4.2	3.7	3.7	3.7	3.7
7	5.7	5.7	5.3	4.9	3.8	3.7	3.7	3.7	3.7
8	5.7	5.7	5.5	5.2	4.3	3.7	3.7	3.7	3.7
9	5.7	5.7	5.3	5.1	4.2	3.9	3.8	3.8	3.8
10	5.7	5.8	5.9	4.6	3.7	3.7	3.7	3.7	3.7
Av. pH ...	5.7	5.7	5.6	5.0	4.0	3.7	3.7	3.7	3.7

yeast, in the usual manner, but no flies were put in. The bottles were kept in an incubator at 25° C. A determination of the pH was made shortly after the bottles were cool and at 24-hour intervals thereafter for eight days. The results are given in Table 2. The initial determinations of pH, made just after the bottles were cool, gave the same reading, 5.7 for all cultures. This was slightly higher than the initial reading of 5.3 found for banana agar by Pearl and Penniman and confirmed by Darby. At the end of 24 hours no change had occurred. By the end of 48 hours the pH had dropped

very slightly to an average of 5.6. At the end of three days the average pH had dropped to 5.0 and during the next day, the fourth, it made a very large drop to 4.0. By the end of the fifth day it had fallen slightly more to 3.7, a value which was maintained to the termination of the observations at the end of the eighth day.

It is to be noted that the degree of resemblance between the different cultures was very high indeed throughout the course of the observations.

In five cultures (2, 5, 7, 8, 9) mold developed, but this apparently made no difference. The lowest final reading (3.6) and the highest final reading (3.8) occurred in two of the moldy bottles.

THE pH OF MEDIUM A, WITH FLIES

The effect of the presence of flies and developing larvae on the pH of the culture medium was investigated. Twelve culture bottles were made up containing 50 cc of medium A. As soon as they were cool a pair of 3-day-old flies were put in each, and the cultures were incubated at 25° C. The determinations were made as before, but the course of the cultures was followed for a longer time, for eighteen days altogether. The parental flies were left in the cultures during the first ten days. The flies of the offspring generation were removed from the cultures as they emerged, but were not counted.

As shown by the means in Table 3, the pH of the bottles with flies began to drop at once and reached the pH levels of 5, of 4.5 and of 4, respectively, about a day and a half sooner than the cultures without flies. The low level reached and maintained was 3.7, exactly as in the case of the cultures without flies. But by the end of the 10th day the pH had risen slightly to 4, and at the end of eighteen days it had reached the value 4.8, by a slow graded rise.

Since the difference in pH early in the cultures had been determined from experiments run successively rather than simultaneously, and since the second experiment had shown a pH rise in the cultures at a period

TABLE 3
MEDIUM A, WITH FLIES; PH READINGS ON SUCCESSIVE DAYS

No.	0	1	2	3	4	5	6	8	10	14	16	18
1	5.8	5.8	4.7	3.8	3.7	3.7	3.7	3.8	4.4	4.8	4.9	4.8
2	5.8	5.6	4.7	3.9	3.6	3.6	3.6	3.6	3.8	4.8	5.0	4.8
3	5.8	5.3	4.6	4.0	3.8	3.7	3.6	3.7	3.9	4.8	5.0	4.8
4	5.8	5.6	4.4	3.7	3.5	3.5	3.5	3.5	3.7	4.2	4.4	4.6
5	5.8	5.6	4.6	3.9	3.8	3.8	3.8	3.8	4.6	4.8	4.8	4.8
6	5.8	5.6	4.4	3.7	3.5	3.4	3.4	3.6	3.6	4.1	4.2	5.0
7	5.8	5.7	4.7	3.9	3.8	3.7	3.7	3.7	4.4	4.4	4.6	4.8
8	5.8	5.5	4.6	3.8	3.7	3.7	3.7	3.8	4.0	4.5	4.9	4.8
9	5.8	5.5	4.0	3.6	3.6	3.7	3.7	4.0	4.0	4.1	4.1	4.6
10	5.8	5.8	4.6	3.9	3.6	3.8	3.9	4.0	4.1	4.8	5.0	4.6
11	5.8	5.6	4.5	3.8	3.7	3.6	3.6	3.8	3.8	4.6	4.7	4.9
12	5.8	5.6	4.6	3.8	3.6	3.6	3.6	3.6	3.8	4.2	4.4	4.6
Av. pH	5.8	5.6	4.5	3.8	3.7	3.7	3.7	3.7	4.0	4.5	4.7	4.8

later than was covered by the first experiment, it seemed advisable to run a small-scale experiment to check these two points.

The cultures were made as before, using 50 cc of medium A. In half of the bottles pairs of 3-day old flies were put and were allowed to remain 5 days only.

As shown by means of Table 4 the fall through succes-

TABLE 4
MEDIUM A; WITHOUT FLIES, CULTURES 1-4; WITH FLIES, 5-8; PH READINGS ON SUCCESSIVE DAYS

No.	0	1	2	3	4	6	8	10	12	14	Flies
1	5.7	5.7	5.4	5.1	4.0	3.7	3.7	3.7	3.7	3.7	
2	5.7	5.8	6.0	5.4	4.6	3.7	3.6	3.6	3.6	3.6	
3	5.7	5.7	5.6	4.3	3.6	3.8	3.9	4.0	4.0	4.0	
4	5.7	5.7	5.6	5.0	4.3	3.7	3.7	3.7	4.2	4.2	
Av.	5.7	5.7	5.7	5.0	4.1	3.7	3.7	3.8	3.9	3.9	
5	5.7	5.4	4.6	3.9	3.6	3.6	3.7	4.2	4.2	4.4	38
6	5.7	5.5	4.5	3.8	3.5	3.5	3.5	3.6	3.6		60
7	5.7	5.6	5.0	4.0	3.6	3.6	3.7	3.7	3.6	3.6	91
8	5.7	5.6	5.3	4.5	3.6	3.6	3.6	3.8	3.8	4.0	95
Av.	5.7	5.5	4.9	4.1	3.6	3.6	3.6	3.8	3.8	4.0	71

sive pH values to the minimum pH occurred slightly more than a day earlier in the cultures containing flies than in the cultures free from flies. Also the slight rise late in the cultures occurred in the cultures free from flies as well as in the cultures with flies.

The rightmost column of Table 4 gives the number of flies that hatched during a 4-day count of the progeny.

MEDIUM A, WITH KH_2PO_4

The effect produced upon medium A by the addition of primary potassium phosphate was tested as follows: Cultures 1 to 10, Table 5, were made as a control, using

TABLE 5

MEDIUM A; CULTURES 1-10; WITHOUT FLIES; CULTURES 11-16, WITHOUT FLIES, WITH KH_2PO_4 ; CULTURES 16-25, WITH FLIES, WITH KH_2PO_4

No.	1	2	3	4	5	7	9	11	13	16	18	20	Flies
1	5.9	4.2	4.0	3.8	3.6	3.8	4.3	4.4	4.4	4.3	3.8		
2	6.0	4.1	3.9	3.6	3.6	3.8	4.0	4.1	4.3	4.2	4.2		
3	5.9	4.0	4.0	3.9	3.6	4.1	4.1	4.2	4.2	4.4	4.5		
4	6.0	4.0	4.0	3.8	3.9	3.9	4.1	4.3	4.3	4.3	4.3		
5	6.0	4.1	4.0	3.8	3.8	3.8	4.2	4.0	4.2	3.8	4.2		
6	6.0	4.2	4.0	3.6	3.8	3.8	4.1	4.1	4.4	4.1	4.4		
7	5.9	4.1	3.9	3.8	3.8	4.0	4.1	4.0	4.2	4.2	4.3		
8	6.0	4.3	4.2	4.0	3.7	3.8	3.9	4.4	4.4	4.1	4.1		
9	5.9	4.1	3.9	3.7	3.8	3.8	4.1	4.2	4.1	4.0	4.2		
10	5.9	4.0	4.0	3.8	3.7	3.7	4.0	4.2	4.2	4.2	4.1		
Av.	6.0	4.1	4.0	3.8	3.7	3.9	4.1	4.2	4.3	4.2	4.2		
11	5.4	4.0	3.7	3.7	3.8	4.0	4.1	4.1	4.1	4.1	4.1		
12	5.5	4.0	3.8	3.8	3.8	4.4	4.2	4.1	4.1	4.1	4.1		
13	5.4	3.9	3.8	3.8	3.8	4.1	4.4	4.1	4.1	4.1	4.1		
14	5.5	4.0	3.8	3.7	3.7	4.2	4.3	4.2	4.2	4.1	4.1		
15	5.4	4.0	3.8	3.8	3.8	4.2	4.2	4.2	4.1	4.1	4.1		
Av.	5.4	4.0	3.8	3.8	3.8	4.2	4.2	4.1	4.1	4.1	4.1		
16	6.0	4.1	4.0	3.7	4.0	4.0	4.0	3.9	3.9	4.0	4.0	4.0	141
17	6.0	4.0	4.0	3.8	3.8	4.0	4.0	3.9	4.0	4.2	4.1	4.2	165
18	5.9	4.1	4.0	3.7	3.7	4.0	4.0	3.9	4.0	4.0	4.0	4.1	151
19	5.9	4.2	3.9	3.8	3.9	3.9	4.0	3.9	4.0	3.9	4.1	4.1	186
20	6.0	4.1	4.0	3.7	3.7	3.8	3.9	3.8	4.0	3.8	4.0	4.0	223
21	6.0	4.0	3.9	3.8	3.6	4.0	3.8	3.8	3.9	3.9	4.0	4.0	303
22	5.9	4.0	4.0	3.8	3.8	3.9	4.1	3.9	4.0	4.1	4.3	4.3	189
23	6.0	4.1	3.8	3.6	3.8	4.0	4.1	4.0	4.0	4.0	4.1	4.0	151
24	6.0	4.0	4.0	3.7	3.8	4.0	4.1	4.1	4.2	4.1	4.4	4.2	124
25	6.0	4.1	4.0	3.7	3.6	4.1	4.1	4.1	4.2	4.2	4.1	4.0	89
Av.	6.0	4.1	4.0	3.7	3.8	4.0	4.0	3.9	4.0	4.0	4.1	4.1	172

unmodified medium A, without flies. As a second control, cultures 11-15 were run simultaneously, without flies but with the addition of 0.2 gm KH_2PO_4 per culture. Finally, in cultures 16-25, run simultaneously with the others, medium A, modified by the addition of 0.2 gm KH_2PO_4 per culture, was used with pairs of three-day old flies from the Florida wild stock. Eggs were laid for nine days and the emerging offspring were counted for ten days only. The pH determinations were continued relatively long, for eighteen or twenty days.

Within each set in Table 5 the variability in pH from culture to culture was small. The most striking fact about the three sets is their remarkable similarity to each other. The only difference made by the relatively small amount of phosphate would seem to have been a slight lowering of the initial pH. In contrast to the results in the previous two experiments, the set without flies showed as rapid a drop in pH as did the set with flies. Moreover, all three curves fell to approximately the minimum pH in about two days instead of the three to five days shown previously. The specific factor that differed in the preparation of the early and later cultures is not known. It might be any factor that gave the yeast an unusually good start, such as being more thoroughly distributed over the surface instead of being applied as a single drop. It seemed possible that the difference found earlier between the cultures with and without flies was a result of the rapid distribution of yeast by the feet of the flies and by the crawling young larvae.

The slight rise in pH following the first minimum was present in all three sets. It may be surmised that this rise was due to the production of ammonia or other waste products of alkaline tendency by the growing yeasts and larvae.

The number of offspring in the phosphated cultures averaged 172, or if culture 25 be omitted (abnormal in that flies began emerging several days later than in the others), 182. This value of 182 flies per culture seemed

higher than the average for normal unphosphated cultures, as seen in routine work.

EFFECT OF PHOSPHATE ON PRODUCTIVITY, MEDIUM A

The buffering effect of the phosphate on the pH of the medium had been negligible in this pH range and in the amount used. But phosphates have other effects, such as modifying sugar metabolism, and it was thought such effects had been important here. Accordingly, two sets of medium-A cultures were run simultaneously, both with three-day flies from Florida wild stock. One set, cultures 1-9, Table 6, served as control and the other cultures (10-18) were modified by the additions of 0.2 gm KH_2PO_4 per culture. The parents remained in the bottles for nine days, and the counts of the offspring were continued for nine days only. The parents in cultures

TABLE 6
CULTURES 1-9, MEDIUM A; 10-18, MEDIUM A, WITH KH_2PO_4 .

No.	1	3	5	9	11	13	15	Flies
1 ...	5.5	4.4	3.6	3.6	3.8	4.1	4.3	125
2 ...	5.6	4.4	3.6	3.6	3.6	3.6	3.8	165
3 ...	5.6	4.4	3.6	3.6	3.6	3.6	3.6	136
4 ...	5.6	4.7	3.6	3.7	3.6	3.6	3.7	212
5 ...	5.6	4.1	3.6	3.6	3.6	3.6	3.6	170
6 ...	5.6	4.7	3.6	3.6	3.6	3.6	3.6	131
7 ...	5.7	4.4	3.7	3.7	3.8	4.0	4.0	83
8 ...	5.6	4.4	3.6	3.8	3.7	4.0	4.0	119
9 ...	5.6	4.5	3.6	3.7	3.7	3.6	3.6	124
Av.	5.6	4.5	3.6	3.7	3.7	3.8	3.9	142
10 ...	5.5	3.7	3.6	3.6	3.8	3.8	3.8	267
11 ...	5.6	3.7	3.7	3.8	3.8	3.8	3.8	241
12 ...	5.5	3.7	3.6	3.6	3.8	4.0	3.9	215
13 ...	5.4	3.6	3.5	3.6	3.7	3.7	3.8	284
14 ...	5.5	3.9	3.7	3.6	3.7	3.8	3.8	228
15 ...	5.5	3.7	3.7	3.9	3.9	4.0	4.0	198
16 ...	5.4	3.7	3.3	3.8	3.8	3.8	3.8	229
17 ...	5.5	3.9	3.7	3.9	3.7	3.8	3.8	183
18 ...	5.4	3.9	3.7	3.8	4.0	4.0	4.0	212
Av.	5.5	3.7	3.6	3.7	3.8	3.9	3.9	229

1 and 7 died early. The average progeny for the remaining seven normal cultures was 151. The average number of offspring in the cultures modified by the addition of phosphate was 229, or half again as many in the control cultures. The presence of the phosphate seems therefore to have had again a favorable effect upon the productivity, and to have decreased variability.

The curve for the means of the pH determinations of the buffered cultures is practically identical with that of the control cultures, except for the lowered pH value early in the course of the experiment.

SOME DEVELOPMENTS OF THE CORNMEAL MOLASSES AGAR METHOD

“Brer Rabbit” molasses, in common with nearly all the commercial brands of molasses easily available, was characterized by the presence of SO_2 . The SO_2 may have been used to make the color lighter, or may have been used to check the fermentation that occurs in the less heavy grades of molasses. That this SO_2 would likewise check the growth of yeast in the culture bottles seemed likely. Accordingly, a supply of molasses of high sugar content and entirely free from SO_2 was secured from a wholesaler.

In testing this heavy molasses as a substitute of the former mixture of molasses and “Karo” the amount was decreased, since it seemed that the old formula was unnecessarily high in carbohydrate and relatively low in nitrogen supply. The cornmeal was increased slightly to augment the nitrogen but could not be increased much more without making the medium crumbly under the action of the larvae. The first formula tried was the following:

Medium B	Water	70.0 cc.
	Agar	1.2 gm.
	Cornmeal	17.0 gm.
	Molasses	11.8 cc.

Seven cultures made up according to this formula (Table 7) gave pH readings more favorable than the old formula in that the minimum was about 4.0 rather than 3.7, and was consequently nearer the optimum pH for yeast growth, namely, 4.5. The parents (all experiments employed the Florida stock) were left in the cultures for a ten-day laying period. The offspring, counted for ten days of emergence, averaged 266, higher than the 151 of medium A and than the 182 and 229 of phosphated medium A. Part of this increase was presumably due to the fact that the amount of media per culture was increased from 50 cc to 60 cc. The cultures were entirely free from molds, and in general characteristics seemed preferable to those using the older formula.

TABLE 7
MEDIUM B

No.	Days			Flies
	1	3	7	
1	5.8	4.9	4.0	191
2	5.7	4.9	4.0	292
3	5.8	5.0	4.1	273
4	5.8	4.9	4.0	334
5	5.8	5.0	3.9	320
6	5.7	4.9	4.1	272
7	5.8	5.0	4.0	277
Av.	5.8	4.9	4.0	266

TABLE 8
MODIFIED MEDIUM B

No.	1	3	7	Flies
1	4.9	4.3	4.3	208
2	4.9	4.3	4.4	
3	5.0	4.4	4.4	33
4	4.9	4.3	4.2	341
5	4.9	4.3	4.4	144
6	5.0	4.3	4.3	
7	4.9	4.4	4.4	289
8	4.9	4.3	4.3	358
9	5.0	4.3	4.4	464
Av.	4.9	4.3	4.3	204

MEDIUM B, MODIFIED BY TARTRATE AND PHOSPHATE

The culture media used for yeasts are usually provided with nitrogen in the form of an ammonium salt. Tartrates are also used and have a buffering action. We therefore ran a test series (Table 8) in which culture medium B was modified by the substitution of one gram of ammonium tartrate for one gram of the cornmeal. A small amount (0.1 gm per hundred parts) of KH_2PO_4 was also added.

The pH was initially relatively low (4.9) and its minimum was relatively high (4.3), both deviations being in the direction of stabilizing at the optimum pH for yeast growth. There was an exceptionally heavy growth of yeast in all the cultures. But the production of offspring was very erratic, both in totals and in time of emergence. Two of the cultures produced no flies, although very large numbers of small larvae had been present and many had pupated as small pupae. In another culture 33 small pale flies were produced from large numbers of small larvae and pupae. On the other hand, three cultures produced the unusually large number of about 300 flies each and one produced 464 flies, although these also were rather small and pale. It was supposed that the ammonium tartrate had been used in too great a concentration and had exercised an unfavorable direct effect upon the larvae in addition to the apparently highly favorable effect upon the yeast.

MEDIUM C, PLAIN AND MODIFIED

On account of the great irregularity in the above result, the test was repeated, but with slight changes in the formula. The amount of ammonium tartrate was reduced to half the previous amount (to 0.5 gm per 100 parts of the medium). "Quaker Oats" cornmeal was used in medium C instead of the usual chicken-feed cornmeal. "Quaker Oats" cornmeal, intended for use as a breakfast cereal, has been very carefully sifted as to size of granule and portion of the grain used and is a much more uniform product than the ordinary cornmeal, which is the mixture of various sized granules and flourlike dust which comes directly from the grinders. "Quaker Oats" cornmeal makes a much stiffer starch paste when it sets, and accordingly the percentage of agar was lowered to 1 instead of the 1.2 that had been used in the former series.

The nine cultures using plain medium C (Table 9) showed an initial pH of 5.8 and a minimum apparently

not far from the optimum of 4.5. The cultures uniformly produced very high outputs, averaging 308, of large flies. The parents had been in nine days, and the offspring were counted for ten days' emergence.

In the nine cultures modified by the ammonium tartrate and the phosphate (Table 10) the pH was apparently the same as in the plain medium C. The output averaged practically the same (296), but most of the cultures were slightly lower, while two had unusually high progenies of over 500 flies. Hence the results of the modification with ammonium tartrate were again irregular, but showed the possibility of marked benefit.

MEDIUM D AND SOME MODIFICATIONS

Some tests just completed by Mr. M. Harnley (1929) on the banana agar medium had shown that greater numbers of offspring were produced when the amount of agar was increased above the 1.0 that had been standard for years. Accordingly, in the next set of tests, in November, 1927, the agar concentration was raised to 1.4 per cent. Four series of cultures with flies were run, using the medium with higher agar content (medium D) and some modifications of it.

	D	D-1	D-2	D-3
Water (cc)	70.0	70.0	70.0	70.0
Agar (gm)	1.4	1.4	1.4	1.4
Cornmeal (gm)	17.0	17.0	17.0	17.0
Molasses (cc)	11.6	11.5	10.6	11.1
KH ₂ PO ₄ (gm)	0.1
(NH ₄) ₂ C ₄ H ₄ O ₆ (gm)	1.0
HNO ₃ (gm)	0.5
Total parts	100.0	100.0	100.0	100.0

In Table 11 are given the results of the 10 cultures in which medium D was employed. Great care was used to have the different cultures precisely alike initially. The pH was uniformly 5.8 after cooling and 5.7 at the end of one day. The fall was rapid on the second and third

TABLE 9
MEDIUM C

No.	0	1	2	3	4	9	Flies
1	5.7	5.7	5.2	4.6	4.5	4.0	352
2	5.8	5.7	5.2	4.6			350
3	5.8	5.7	5.0	4.5		4.0	221
4	5.8	5.7	5.1	4.8	4.8		296
5	5.8	5.7	5.2	4.5			283
6	5.8	5.6	5.2	4.6	4.8	4.4	346
7	5.8	5.7	5.2	4.5	4.6		271
8	5.8	5.7	5.2	4.3			264
9	5.8	5.7	5.2	4.6	4.6	4.1	390
Av.	5.8	5.7	5.2	4.6	4.6	4.1	308

TABLE 10
MEDIUM C, MODIFIED

No.	0	1	2	3	4	9	Flies
1	5.8	5.7	4.3		4.2	4.3	507
2	5.8	5.6	4.4				252
3	5.8	5.7		4.4			243
4	5.8	5.7	4.6	4.6	4.6	4.6	191
5	5.8	5.6			4.2		213
6		5.7					228
7	5.8	5.6			4.0	4.0	258
8	5.8	5.7		4.4	4.1		559
9	5.8	5.7	4.6			4.1	215
Av.	5.8	5.7	4.5	4.5	4.2	4.2	296

days, to the minimum of 4.1 on the fourth day. The readings were not continued after it was seen by the fifth day's results that the minimum had been reached.

Into each bottle was put, shortly after the cooling and the initial determination of the pH, a pair of two-day-old flies from the Florida wild stock. They were allowed to remain six days. Only in the case of culture 3 had the female perished some time before the removal. The offspring were counted daily as they emerged. It had been intended to continue the counts for ten days, but after eight days of counting the heat control in the incubator

TABLE 11
MEDIUM D

No.	0	1	2	3	4	5	Flies
1	5.8	5.7	5.2	4.6	4.1	4.1	383 (292)
2	5.8	5.7	5.2	4.5	4.2	4.1	341 (259)
3	5.8	5.7	5.2	4.7	4.2	4.2	226 (212)
4	5.8	5.7	5.2	4.4	4.1	4.1	363 (288)
5	5.8	5.7	5.2	4.5	4.1	4.1	401 (310)
6	5.8	5.7	5.1	4.5	4.1	4.1	286 (241)
7	5.8	5.7	5.4	4.6	4.2	4.1	434 (332)
8	5.8	5.7	5.2	4.6	4.1	4.2	408 (345)
9	5.8	5.7	5.2	4.5	4.1	4.1	332 (310)
10	5.8	5.7	5.1	4.5	4.1	4.1	289 (229)
Av.	5.8	5.7	5.2	4.5	4.1	4.1	336 (282)

TABLE 12
MEDIUM D-1

No.	0	1	2	3	4	5	Flies
1	5.8	5.6	5.3	4.4	4.2	4.2	295
2	5.8	5.6	5.2	4.4	4.2	4.2	201
3	5.8	5.6	5.2	4.3	4.2	4.2	195
4	5.8	5.6	5.2	4.4	4.2	4.2	266
5	5.8	5.5	5.2	4.4	4.2	4.2	235
6	5.8	5.6	5.2	4.5	4.3	4.3	259
7	5.8	5.6	5.2	4.4	4.2	4.2	198
8	5.8	5.6	5.2	4.3	4.2	4.2	196
9	5.8	5.6	5.2	4.4	4.2	4.2	199
10	5.8	5.6	5.2	4.6	4.2	4.2	247
Av.	5.8	5.6	5.2	4.4	4.2	4.2	230

failed and all further flies were killed. The average for the eight days was 336, or of the nine normal cultures, 349. Since the egg laying was only 6 days instead of ten and the counting period eight days instead of ten, the average of 336 or, more accurately, 349, must be considered remarkably high. The best previous series (Medium C, Table 9) had given an average of 308 for ten and ten as periods for egg laying and emergence. In parentheses in the final column of Table 11 are given totals for the first six days of emergence, for comparison with series D-1 and D-2 which were cut short after six days' emergence, by the same incubator failure. The six days' average for ten cultures was 282, or 289 for the nine normal cultures.

In Table 12 are given the results of similar cultures, using medium D-1 (0.1 gm KH_2PO_4). The pH readings were little different from the plain D results. The emergence period was limited to six days by incubator failure. The average for all ten cultures was 230, or 233 for the nine cultures in which the female was alive and normal at the end of the six days' egg laying period (*i.e.*, excluding culture 7). This value of 233 is distinctly lower than the comparable value of 289 for plain medium C. Evidently the presence of the phosphate had not improved conditions here but rather the reverse.

TABLE 13
MEDIUM D-2

No.	0	1	2	3	4	5	Flies
1	5.8	4.9	4.7	4.5	4.2	4.1	186
2	5.8	5.2	4.8	4.5	4.2	4.0	194
3	5.8	5.2	4.8	4.5	4.2	4.1	126
4	5.8	4.9	4.7	4.5	4.3	4.1	276
5	5.8	5.2	4.7	4.5	4.3	4.1	167
6	5.8	5.0	4.8	4.5	4.2	4.1	122
7	5.8	4.9	4.8	4.5	4.2	4.1	276
8	5.8	5.0	4.7	4.5	4.1	4.0	354
9	5.8	5.0	4.6	4.5	4.2	4.0	87
10	5.8	5.2	4.7	4.6	4.3	4.1	282
Av. ...	5.8	5.1	4.7	4.5	4.2	4.1	206

TABLE 14.
MEDIUM D-3

No.	0	1	2	3	4	5	Flies
1 ..	5.8	5.7	5.5	4.8	4.8	4.8	347 (275)
2	5.8	5.7	5.4	4.8	4.8	4.8	308 (265)
3	5.8	5.7	5.4	4.8	4.8	4.8	292 (257)
4	5.8	5.6	5.5	5.2	4.9	4.8	239 (212)
5	5.8	5.7	5.4	5.0	5.0	4.9	397 (335)
6	5.8	5.7	5.5	4.9	4.8	4.8	312 (251)
7	5.8	5.7	5.4	4.8	4.8	4.8	366 (293)
8	5.8	5.6	5.5	4.8	4.8	4.8	337 (302)
9	5.8	5.8	5.5	4.9	4.9	4.8	367 (319)
10	5.8	5.6	5.5	4.9	4.9	4.9	355 (299)
Av. ...	5.8	5.7	5.5	4.9	4.8	4.8	332 (281)

In Table 13 are given the results of tests of medium D-2, modified by the presence of ammonium tartrate. The pH readings were practically identical with those of the plain and of the phosphated media. The offspring were recorded for six days only because of the failure of the incubator on the seventh day. In cultures 5 and 6 the female died after 4 days of egg laying. The other cultures were from 6 days egg laying. The emergence of offspring was delayed one day in three cultures (4, 7 and 10); two days in one culture (3); and three days in another (9). The numbers of offspring fell off early in two cultures (5 and 6), and in these the mothers were known to have died early. The resulting progenies showed a range of from 87 to 354 offspring, with an average of 206 for all, or 221 for the eight normals, excluding 5 and 6. This average was even lower than that for the series raised on medium D-1, modified by KH_2PO_4 , and there was far greater variability and unevenness in the results. A relatively low modal production and a high variability had likewise characterized the results of both previous tests of the effect of ammonium tartrate.

In Table 14 are given the results of a trial of medium D-3 with a richer nitrogen supply in the form of KNO_3 . Here the pH readings changed the least of any observed, reaching a minimum of 4.8. The egg laying was for six days and the counting for eight days. Tests of D-3 were begun simultaneously with D and likewise of D-1 with D-2; all ended simultaneously because of the incubator failure. The female of culture 4 was injured in the taking of samples for pH determinations, and thereafter walked about sluggishly with the wings curled together above her back. Her low output (239 or 212) was presumably due to this injury. The other nine cultures gave an average of 342 flies for eight days' emergence and of 299 for six days' emergence. This record of 299 was slightly better than the comparable 289 for plain medium D, and the results were more uniform, ranging from 251 to 335 instead of from 229 to 345. The KNO_3 was thus

no detrimental factor but was possibly a slightly favorable modification.

ROUTINE MEDIUM, 1928

With the failure of the incubator, the series of tests was broken. However, the two most important improvements, the elimination of SO_2 and other preservatives from the molasses and the rise in the percentage of agar, were incorporated in the routine procedure for making cultures for the stocks of *Drosophila* and for the experiments. The composition of the medium was further changed by lowering the content of the cornmeal and increasing that of molasses and water. These changes were for greater ease in preparing the media (pouring, etc.) and to reduce the granulation of the surface under the action of many larvae. The formula was as follows:

	Water	71.6
	Cornmeal	14.0
Medium 1928	Molasses	13.0
	Agar	1.4

DETERMINATION OF pH OF BUFFERED CULTURES BY THE QUINHYDRONE ELECTRODE

With the above medium as a basis some experiments on buffering were made by Mr. Albert Tyler, to whom we are indebted for the following account.

The pH measurements were made with the quinhydrone electrode, which was preferable in that it was not poisoned by the media.

The amount of buffer to be used was determined by weighing out a sample of the media, taken from a 16-day-old culture, getting its E.M.F. in the set-up and then triturating in the buffer until the E.M.F. reached a value which corresponded with the pH of 4.0. Cultures were then made up, using the calculated amounts of acetate, phosphate, tartrate and citrate buffers. None of these gave offspring and the parents died. When amounts were used to give buffer action up to twelve days, the

same results were obtained. The amounts finally used corresponded to the acidity at the end of about six days.

In none of the sets of cultures was the output of flies greater than in the controls of plain medium 1928. In all there was greater loss of cultures due to death of parents and to heavier growth of molds. In some cultures to which acetic acid was added the few flies were all very small. The phosphated medium gave flies larger than normal but not more numerous than in the controls. The pH maintained the highest level, throughout the nineteen days of the determinations, in the cultures to which only sodium acetate had been added. The number of cultures (5) used in any one test was too small to give more than a rough indication of the situation.

CALIFORNIA ROUTINE MEDIUM

On account of the drier climate of California it was found advisable to still further increase the water content to withstand the greater evaporation. This was accompanied by a decrease in the proportionate amount of cornmeal and an increase in the total food to 60 cc per culture. The formula in routine use at the laboratories of the California Institute of Technology during the fall of 1928 and continuously to the present date is as follows:

	Water	75.0
	Cornmeal (chicken feed)	10.0
California medium	Molasses ("Grandma's")	13.5
	Agar	1.5

In preparing this food the cornmeal is first soaked in enough of the water to wet it thoroughly and leave an excess of water after all the cornmeal is stirred in smoothly. The agar is then put to heating in about two thirds of the remaining water. As the water and agar come to a boil more of the water is added with stirring. This method of adding the water hastens the solution of the agar. After solution of the agar the molasses is stirred in and then the wet cornmeal. After the whole is brought to a boil and kept boiling for about seven

minutes the remaining water (about a tenth of the whole water) is stirred in. The medium is then ready to be poured into the sterilized half-pint culture bottles by means of a large funnel and hose controlled by a large spring clamp. The medium should be poured about one inch deep in the bottles. As low as three quarters of an inch will do, especially for stock cultures, but the greater depth will give a greater output and better ratios for weak classes of offspring. The medium should be made up in fairly large lots, 50 to 200 bottles. A reserve of bottles can be kept for two or three days in a cold room or refrigerator, though this is not advisable if bottles can be made fresh as required.

DETERMINATION OF pH BY BLOCK COMPARATOR

The testing of the pH of the California routine medium was undertaken by Mr. N. K. Schaffer at the California Institute of Technology. He employed La Motte color standards and La Motte 0.4 per cent. standardized indicator solutions. Instead of the drop method employed by Darby, Schaffer used test-tubes and a block comparator, as described by Clark (1928).

In the tests carried out in March, 1929 (Table 15), the pH values differed from the previous findings for cornmeal molasses agar media only in the slower fall to the minimum pH of 3.7 in the case of cultures without flies and 3.6 for the cultures with flies. The output was low, 159 for six cultures that produced consistently, and 0, 5, 11 and 41 for four others. The failures and the low productivity do not constitute a fair representation of the food medium, since a wild stock was used (Oregon R) which is now known to behave erratically.

Four years' experience with this medium has shown that it is excellent, certainly more satisfactory than any previously used in routine work, and relatively inexpensive. Molds are practically unknown unless a culture has already failed from sterility. Molds may develop if cultures are kept very long. Hence it is advisable to

discard all cultures as soon as counts are finished or a culture is found to be sterile. Discarded cultures should be washed up at once and not allowed to stand about growing crops of molds (and perhaps also mites). This formula has been used rather extensively in other laboratories also, and while we have no detailed reports on it, it seems to have been acceptable.

STERN'S FORMULA

Dr. C. Stern was working in the Columbia Laboratory at the time of changing over from the banana agar to the cornmeal molasses agar method. In a recent publication (1929), Stern gives the following formula of the medium in current use in the Kaiser Wilhelm Institute.

	Water	2125		Water	600
A	Molasses	375	B		
	Agar	30		Cornmeal	400

Boil mixture A until the agar has dissolved, then stir in mixture B and continue heating until thickening has occurred.

EFFECT OF pH ON PUPATION

After the foregoing researches were completed, one of the writers (Darby) began some researches on the pupation of *Anastrepha ludens* (Loew), the Mexican fruit fly, in the laboratory of the U. S. Bureau of Entomology in Mexico City. The larvae of this fly, when full grown, leave the fruit in which they have passed their larval life, burrow into the soil, going some distance until clear of the acids produced by the decaying fruits. Sometimes they are forced by circumstances to pupate within the fruit, and in these cases the pupal shell is exceedingly thin. The acidity of the fruit, guava in particular, drops to pH 3.0 and is therefore equal to the acidity of Pearl's cultures. This recalls the fact that in very acid bottles *Drosophila* larvae generally climb up the paper out of the acid medium and may even go as far as the stoppers. If the acidity were forced up to pH 3.0, as suggested by

Pearl, this factor would become important in the formation of viable pupae.

SUMMARY AND CONCLUSIONS

A general survey of the results of the foregoing experiments on various cornmeal molasses agar media suggests the following conclusions: With respect to pH there was uniformly a very high similarity between the several cultures of a set. The different sets of cultures that were similar in formula and treatment, but made up at different times, showed definite but not very large differences in the initial pH, in the length of time taken to reach the minimum, in the value of this minimum and in the amount of subsequent rise.

All sets prepared from unbuffered media showed essentially the same type of curve—an initial pH value of about 5.8 (5.6–6.0) which was maintained for one or two days, and a rapid fall occupying one to three days, usually two, to a minimum of 3.6 to 3.8. This minimum was maintained about three days, and was followed by a gradual rise of slight amount (0.3 to 1.0), reaching a nearly uniform condition at a pH of 3.8 to 4.8. The presence of flies hastened the drop by about one day, possibly by scattering the yeast more thoroughly. The final rise in pH was greater in the cultures containing flies, probably on account of the larger amounts of alkaline waste products from the growing larvae. With unbuffered media the pH during the major portion of the course of the cultures lies below the optimum value for yeast, which is approximately 4.5.

In the media modified by the addition of chemicals having buffering action, the initial pH was most modified, and subsequently the cultures approached in value the controls. In two sets the pH did not drop to a minimum which was as low as elsewhere. In one of these the salt added was sodium acetate, which tends to buffer at a high pH ($4.6 \pm$), relative to the normal minimum of unbuffered cultures ($3.7 \pm$). In the other case, the salt added

was NaNO_3 and it is not clear why this should have kept the pH high, for HNO_3 is not a weakly ionized acid. Since the acidity present in the normal culture is due mainly to acetic acid, it would seem that the simplest and most natural buffer would be Na acetate, especially since it tends to buffer at a more favorable pH than the phosphate does.

With respect to the output of flies, the improvements made raised the output from the initial average of 151 (Table 6) to a high one of 342 (Table 14) for eight days' emergence, equivalent to about 425 for ten days' emergence. This improvement was due principally to removing the harmful SO_2 , to increasing the proportion of agar (1.0 to 1.4) and to increasing the quantity of food (50 cc to 60 cc) per culture. Relatively little favorable effect is attributable to the chemicals added. Phosphate tended to produce larger flies and sometimes to increase their numbers; tartrate tended to increase the variability of the output, with occasional phenomenal outputs; NaNO_3 gave a slightly more favorable effect, but has not been adequately tested.

In view of the relative amounts of variability met with in the tests, it would seem that pH determination on five cultures of a series would give a close enough approximation; while for the fly outputs above twenty cultures should be used.

POSSIBILITIES

It is clear that the following chemical modifiers of the medium offer favorable possibilities that may develop through further trials: sodium acetate to keep the pH from falling too low; potassium phosphate for a directly favorable effect upon the growth processes; an ammonium salt (possibly ammonium nitrate) as a means of increasing the relatively low nitrogen content of the media, and alcohol as a deterrent of other growths than yeast.

The use of cornmeal as a combined food and filler has the objection of being more granular than fibrous in nature, especially when combined with the high agar content that seems desirable. Possible substitutes that may be suggested for trial are: crushed wheat, preferably a gluten wheat; rolled oats, with flat broad plates instead of small round granules; malted barley, with the contained barley hulls; coarsely ground raisins; figs.

Gelatine instead of agar would offer a source of food as well as of mechanical stiffness. But since it is attacked by bacteria, careful tests would be needed to develop a successful method of using it.

Absorbent or toweling paper in small strips can be worked down into the surface of the food, but is considerable labor to prepare. Cotton waste, glass wool or asbestos fibers could be used more conveniently and be more thoroughly incorporated in the food mass. In the spring of 1930 cotton waste was tried and was found to give a very favorable effect upon the surface texture of the medium. The best method seems to be to use the cotton waste in addition to toweling paper. The toweling paper is a double thickness piece two inches wide by four long and is embedded edgewise in the hot food immediately after the pouring and pushed down vertically until its lower edge touches the bottom of the bottle. The cotton is put in after the medium has solidified and been yeasted. A triangular groove about a half inch on the side is cut at one edge of the food mass and extending to the bottom. This channel offers escape for the CO_2 produced by fermentation and keeps the food from being driven up and out of place. This channel is stuffed loosely with cotton waste, using a square-ended narrow-bladed blunt knife. Extra waste is punched into the surface in tufts. The larvae forage preferably about the waste and pupate in the part that rises above the surface. The waste tends to keep the surface from crumbling.

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SHORTER ARTICLES AND DISCUSSION

SINGLE OR MULTIPLE SEX-FACTORS

WHETHER sex is dependent upon a single pair of factors in the so-called sex-chromosomes, or whether its expression is to be regarded as the combined outcome of a number of factors located in the autosomes as well as in the sex chromosomes is a question now actively debated. As is well known, Goldschmidt, from his *Lymantria* work, and Winge, from his work with *Lebistes*, have brought forward strong reasons for taking the former view. On the other hand, the Drosophilists, mainly on the chromosomal conditions accompanying the phenomenon of intersexuality in *Drosophila*, are strongly inclined to the latter view. Quite recently the question has been ably discussed by Bridges.¹ The object of the present note is to offer a brief criticism on some of the evidence on which he takes his stand. In arguing for the multiplicity of sex factors he has made use of the very ingenious work of Dobzhansky and Schultz,² who showed that the grade of intersexuality in *Drosophila* was shifted in the female direction when the flies contained a fragment of an X-chromosome in addition to the usual complement. Further, they claimed that the longer the piece of X-chromosome introduced, the greater was the shift towards the female condition. This, of course, would be a very strong piece of evidence for a multiplicity of sex factors in the X-chromosome, but in going over the data I feel doubtful whether they can bear this interpretation. In the experiments six different types of fragmented X-chromosomes were made use of, and these may be shown schematically in the following figure based on Table 2 in the paper of Dobzhansky and Schultz.

It will be noticed that five of them have in common the "yellow" end of the fragmented X, while one (126) does not contain it. Of the five with the "yellow" end two (107 and 118) are apparently identical.³ 112 is slightly shorter, while L.V.M. is considerably longer. We may now turn to the figures showing the influence of the six duplications in producing a tendency to femaleness as compared with the controls. This was estimated by subdividing the intersexes in both influenced

¹ "The Genetics of Sex in *Drosophila*," *Sex and Internal Secretions*, 1932.

² *Proc. Nat. Acad. Sc.*, xvii, 1931, pp. 513-518.

³ In the text 118 is stated to be longer than 107, but in Table II (p. 515) they are mapped out as being similar.

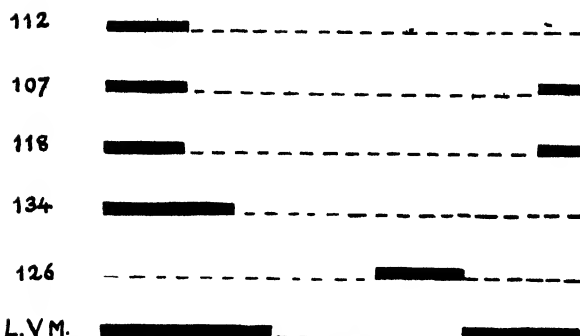


FIG. 1. Schematic representation of the 6 fragmented X-chromosomes. In every case the left end is that containing the locus for "yellow."

and controls into six grades, grade 6 being that nearest to the female type. Table 3 in the authors' paper gives the actual results, but for our purpose we need consider only the summary given in Table 4, which is here reproduced.

TABLE 4
THE MEAN TYPE OF THE INTERSEXES CARRYING THE DUPLICATION AND FREE FROM IT

Duplication	Carrying the duplication	Control	Difference
126	2.74 ± 0.12	2.38 ± 0.21	0.36 ± 0.24
112	4.75 ± 0.055	2.59 ± 0.08	1.56 ± 0.10
107	4.43 ± 0.04	2.43 ± 0.06	2.00 ± 0.07
118	4.53 ± 0.05	1.97 ± 0.075	2.56 ± 0.09
134	4.70 ± 0.07	2.50 ± 0.06	2.20 ± 0.09
L. V. M.	6.0	2.6	3.4

In criticizing the data the points to which I wish to draw attention are as follows:

(1) For 126 the difference between the mean grade of the intersexes carrying the duplication ($=2.74$) and that of the controls ($=2.38$) is only 0.36—a difference less than twice the probable error, and therefore probably not significant. The result is compatible with the supposition that 126 exerts *no* influence on the grade of intersexuality. As already noted, 126 is the only duplication that does not carry the "yellow" end of the X-chromosome. On the other hand, all the other five duplications show a decided influence in shifting the grade of intersexuality towards the female direction. This is compatible with the supposition that a definite female sex factor occurs somewhere in

the "yellow" end of the X-chromosome, and not in the fragmented portion carried by 126.

(2) We may next consider the contention of the authors that the amount of shifting is proportional to the length of the X fragment, and in doing so we shall take account only of the four duplications 112, 107, 118 and 134. For L. V. M. obviously stands apart from the rest. The figures in column 1 of Table 4 show a gradually increasing shift for these four duplications when arranged in order of increased size. Nevertheless, the extremes are separated by .55 of a grade only, and the difference between 107 and 118 is barely significant. When, however, we turn to the controls in column 2 we meet with marked irregularity, the extremes being separated by .64 of a grade. Now if the triploid mothers used were of the same stock, and there is nothing in the account to lead us to suppose otherwise, this variability may well make us hesitate before accepting the apparently orderly increase shown in the figures of column 1. For it may be merely accidental. If on the other hand we are to regard the control figures as in each case an accurate measure of the material upon which the fragmented X works, and to take the figures in column 3 as the significant ones, then the data do not quite bear out the authors' contention. For the effect of the longest fragment 134 is less than that of 118, and shows a barely significant difference from that of 107.

As already noted, the case of L. V. M. is obviously distinct, and it seems reasonable to suppose that some modifying factor is here to be found in this longest fragment which is not present in the others.

And here a brief word on what the authors term "mere modifying factors." They take the view that since such factors show the same quantitative relations as the sex factors proper the distinction between the two classes is an arbitrary one at best, wherein they are opposed to Goldschmidt and others. Over this question of modifiers I incline to think that the two parties are a little at cross purposes. The "modifiers," among whom I would count myself, appear to have this at any rate in common, *viz.*, the view that the modifying factor has no influence on the phenotypic character associated with the factor modified unless this factor be present. There are many modifying factors for shades of flower color, but in so far as color is concerned they exert no influence unless the factor for color itself be present. So with sex. The "modifiers" would say that there is a definite

sex factor⁵ capable of producing a definite effect of itself, but its expression can be influenced by certain modifying factors. In the absence of the sex factor the modifiers would not of themselves produce any effect in so far as the expression of sex is concerned. On the other hand, I gather that in the conception of the Drosophilists sex is the outcome of a number of factors and that no single one of these factors is absolutely essential; though of course if the complement be abnormal the expression of the character will be abnormal also. In this sense there does seem to me to be a real difference between the Drosophilist's conception of sex and that of his opponents. Whether the two can be brought into line, or whether there is a fundamental difference between *Drosophila* and the other creatures hitherto investigated must be left for future work to decide.

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INTERLOCKING AS A DEMONSTRATION OF THE OCCURRENCE OF GENETICAL CROSSING- OVER DURING CHIASMA FORMATION

DURING an examination of chromosome behavior at meiosis in *Lilium regale* a very interesting and exceptional case of bivalent interlocking was observed at diakinesis. One of the bivalents concerned had a loop interlocked with two loops of the other, each side of the loop of the first bivalent passing through one loop of the second.

Figs. 1-6 are untouched photomicrographs of the configuration, taken at gradually descending foci; Fig. 3 being at the critical focus and showing the center chiasma of the second (horizontal) bivalent between the sides of a loop of the first (vertical) bivalent.

At zygotene the constituent chromosomes of the second bivalent must have become paired between the chromosomes of the first one, so giving when pairing was completed a configuration as illustrated in the diagram (Fig. 7). Chiasmata subsequently formed at the points indicated, and one of the end ones of the second bivalent terminalized, so giving rise to the observed diakinesis configuration.

This example of interlocking has an important bearing on the relations between chiasma-formation and crossing-over. If

⁵ Or pair of factors.

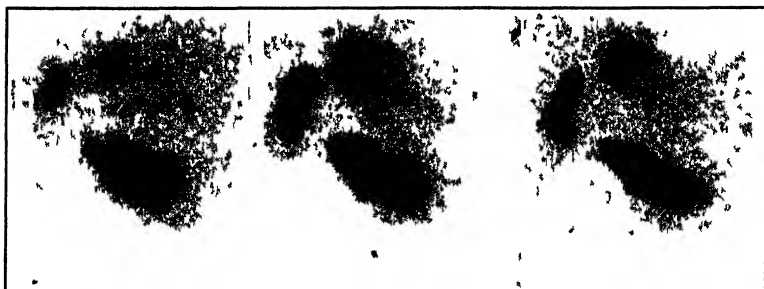


FIG 1

FIG 2

FIG 3



FIG 4

FIG 5

FIG 6

FIGS 1-6 Photomicrographs of the two interlocked bivalents taken at descending foci, Fig 3 shows the critical central chiasma with the interlocked loop of the other bivalent enclosing it (Photos taken by Mr H C Oster stock) \times ca 1400

chiasmata arise by the opening out of reductional and equational loops as the "classical" theory postulates, in one loop of the second bivalent identical chromatids would be on opposite sides of one chromosome (pair of chromatids) of the first bivalent (see Fig 8) This is absurd, as bivalent interlocking must be

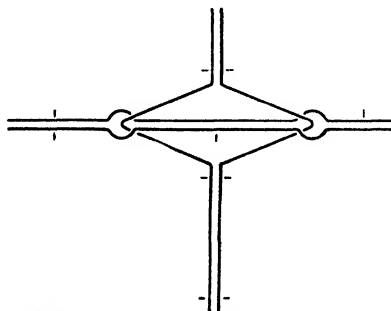
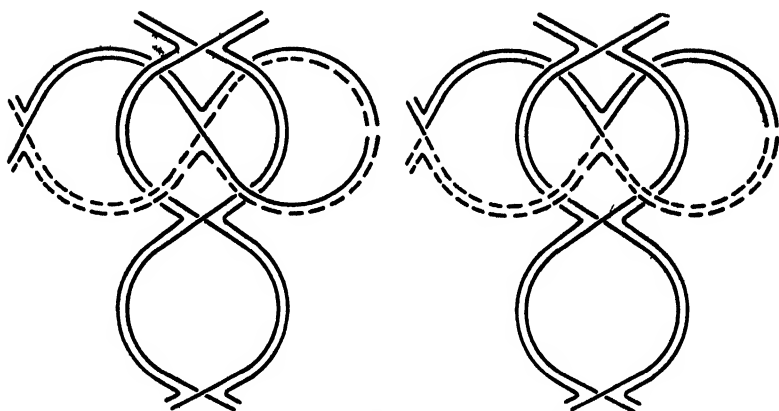


FIG 7 Diagram of the probable mode of pairing of the four chromosomes at pachytene The broken transverse lines indicate the places where chiasmata subsequently arose



FIGS. 8 and 9. Line diagrams of the observed diakinesis configurations. Fig. 8 shows the interpretation on the "classical" theory, leading to identical chromatids being on opposite sides of one interlocked chromosome—an impossibility. Fig. 9 shows the interpretation on the chiasmatype theory.

determined before chiasma formation, *i.e.*, at early prophase before the split of the primary chromosomes into chromatids. Therefore, the center chiasma of the second bivalent must have arisen by genetical crossing-over between two of the four chromatids of this bivalent (see Fig. 9). Thus this case of interlocking is a cytological demonstration of genetical crossing-over at the critical chiasma.

It must be noted that this configuration could have arisen by the opening out of reductional and equational loops if there had been, originally, two chiasmata in the second bivalent between the sides of the interlocked loop of the other bivalent, one of these chiasmata subsequently breaking. Sax (1932) has made a similar postulation with regard to Darlington's demonstration of crossing-over in quadrivalents of tetrasomic *Hyacinthus*. Now in *Lilium*, as in *Hyacinthus*, the reduction in the number of chiasmata from diplotene to metaphase is very small, and so such breakage of a chiasma would be improbable, unless interlocking itself increased chiasma breaking, a postulation which disagrees with the observations of Gairdner and Darlington (1931) on *Campanula persicifolia* and the present author (unpublished) on *Lilium* species. Consequently, it is improbable that such a formation of two chiasmata followed by a breakage of one of them has given rise to the observed configuration.

Hence the exceptional case of interlocking reported here very

strongly supports the chiasmatype theory, as restated by Darlington (1931): "(i) A chiasma is constituted by genetical crossing-over between two of the four chromatids taking part in it, or (ii) association at diplotene is between chromatids derived from the same somatic chromosome."

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A SECOND MOTH FROM THE COLORADO EOCENE

IN the AMERICAN NATURALIST, lxxv (1931), p. 354, the fossil moth, *Chionaemopsis quadrifasciatus* Cockerell and LeVeque, was described from the Colorado Eocene, being apparently the oldest known lepidopterous insect. In the same volume (p. 479), Professor W. T. M. Forbes calls attention to the resemblance of this insect to the genus *Atteva*, belonging to a group apparently connecting the Yponomeutidae and the Pyralids.

Great interest was aroused recently, when Miss Mary L. Moore brought in a second fossil moth from rocks of the same age, the Green River Eocene. It was found by her brother, James Moore, near Rifle, Colorado. In the same collection is an excellent specimen of the previously described *Thamnotettix packardii* Cockerell.

The new specimen represents an entirely different genus and species, and shows the head, palpi and antennae, as well as both wings. It was preserved almost undamaged, the wings still covered with scales and the fringes almost perfect. My first impression was that it was an ordinary Pyralid, perhaps even to be referred to the genus *Pyrausta*; but the venation of the hind wing seems to indicate a member of the family Thyrididae, said by Hampson (1897) to be "closely allied to the ancestral stock of the Pyralidae." The ascertainable characters are as follows:

*Hexerites* NEW GENUS

Small *Pyrausta*-like moths with simple antennae; moderate, porrect, palpi; anterior wings rather narrow, the apical angle greater than a right angle, the tip obtuse; hind wings with the costa very straight, the apical angle rounded, but abrupt and less than a right angle.

Resembles the living (North American) genus *Hexeris* Grote, but the palpi are shorter; the anterior wings are more obtuse, less falcate at end; the outer margin of the hind wings is not at all concave. There is no trace of the characteristic fine lines or bands crossing the wings of *Hexeris*.

Hexerites primalis NEW SPECIES

Palpi rather slender, about 290 μ long; antennae simple, fairly stout, about 65 μ diameter; width of head 960 μ ; anterior wing 6 mm long, lower margin 5 mm, outer margin 2.6 mm; costa straight for over 4 mm, then arched, curved; outer margin quite straight for nearly 2 mm below apex; venation not visible, owing to the heavy scaling; wing uniform light brown, without markings, except that the costa is very narrowly darkened, and the fringe on outer margin is slightly darkened. Hind wing visible in part only, where it appears above the fore wing; it is about 5.3 mm long, pale, with about the apical 2 mm lightly infuscated; whether the base is darkened can not be seen; vein Sc + Rl free at least 2160 μ from its end, where it is slightly curved upward; how much more is free can not be seen because the base is covered by anterior wing; veins Rs and Ml far apart, Rs going to apex of wing. It is the character of Sc + Rl of hind wings which appears to indicate a species of Thyrididae, and in this family the nearest relative appears to be *Hexeris*. I am greatly indebted to my colleague, Mr. Hugo Rodeck, for the photograph. The specimen is in the University of Colorado Museum.

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THE PROGENY TEST AS A MEANS OF EVALUATING THE BREEDING POTENTIALITIES OF FARM ANIMALS

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THE service which genetics may render to agriculture through improvement in the value of farm animals as a source of income to the farmer is of real importance, because it contains the possibility of introducing into agriculture an efficiency in farm operations which will raise the income labor receives on the farm to a par with that of labor in industry. If genetics can successfully perform this service, it will also render a service to itself by broadening the field of its activities and potentialities. To understand how this may be accomplished, it is necessary first to have a clear comprehension of what is required of genetics in this field.

The application of genetics to the improvement of plants and animals of agricultural importance, takes genetics into the field of business, because for the most part agriculture is engaged in producing commodities for the general market which must therefore return a margin of profit to the producer, for without profits no business long survives. Genetics, therefore, if it is to be of service to agriculture, must in some way be able to produce business profits, otherwise agriculture will have no place for it.

¹ Contributed to a symposium on "The Progeny Test as a Means of Evaluating the Breeding Potentialities of the Individual," held by the Genetics Society of America at Atlantic City, N. J., December 27, 1932.

Profits arise in business when the selling price exceeds all costs of production. Since the farmer both buys and sells in a market where prices are fixed by influences beyond his control, he is compelled to seek his profits in the narrow field between costs and selling price; that is, in those operations on the farm over which he exercises some measure of control.

As the great crops of the farm, such as hay, corn, etc., are not produced for human consumption but are mostly fed to farm animals to produce other products better suited to man's needs or desires, the efficiency of farm animals in converting these crops into salable products becomes of great importance. Genetics, then, must introduce greater efficiency into the operations of animal husbandry on the farm, by producing animals of greater productive capacity or greater ability in the conversion of the bulky crops of the farm into products consumed as food by man or otherwise directly serving human needs than those now available.

Thus the improvement of farm animals is a matter of improvement in those qualities which contribute to the livelihood of the farmer, either in his home or through sale in the markets of the country. The progeny test, therefore, must be considered from this standpoint.

Improvement of farm animals—in the minds of many people—deals with wholly non-commercial subjects, such as improvement in type, color and other features which dominate the show ring. In saying that such features are non-commercial, the fact that some people derive an income from operations in characters of this kind has not been overlooked, but the value of the articles they sell depends upon the whim of individuals and will not be found quoted in the daily papers as will the price of butter or wool. They are, therefore, of no real commercial value. Moreover, the world of commerce will not pay a penny more for the products which come from champion show ring animals than from those less famed in this respect. All it cares about is the quality of the goods it buys and sells. For this market, those animals which

most cheaply produce the goods it requires are those most suited for general farming. I shall therefore deal with the progeny test as utilized in the improvement of farm animals solely from the standpoint of commercial usefulness.

At once a question arises in connection with the meaning of improvement. Does it refer to occasional individuals or averages? Clearly averages, because while improved individuals are important as stepping-stones to improved averages, it is only when the average of the race is improved that the improvement comes to have commercial significance. When a dairy cow makes a world's record, it marks no improvement in average dairy cows. Improvement in dairy cattle acquires commercial significance only when the average yield of dairy cattle is increased. The use of the progeny test in the improvement of farm animals has meaning then, only as improvement in averages is brought about.

Having stated these general conditions relating to the progeny test in the improvement of farm animals, we are in a position to consider the test itself. Though often considered to be of comparatively recent origin, perhaps because it is the foundation of the very modern science of genetics, breeders have attached considerable significance to the progeny test for a long time. The word prepotent—used by breeders long before genetics came into being—is indicative of the recognition given progeny as a guide to the breeding value of the individual, but they did not know how to utilize the progeny test, for the true nature of inheritance was still a mystery. It required the genius of Gregor Mendel, by bringing order out of seeming chaos, to make the progeny test the foundation upon which a real science of inheritance could be built. The development of this science thus far has been along the lines of acquiring a knowledge of the processes of inheritance, but, as the program of the Sixth International Congress of Genetics held last summer reveals, very little consideration has as yet been given to the utilization of the knowledge thus gained in the improvement of the

plants and animals on our farms. Here is a great undeveloped field of human endeavor the potentialities of which are fully as great as those in the fields of chemical or electrical engineering.

In the application of genetics to the improvement of agricultural plants and animals, the principles upon which it is established are the best guide in all practical breeding operations. There are, however, numerous modifications of detail which are imposed by differences in the plants and animals concerned, but their number is so great that it is impossible to do more than outline some of the possibilities.

The essence of the progeny test, briefly stated, consists in determining the number and proportion of the various kinds of progeny produced by an individual and from these to derive a statement of its breeding ability. This statement, when reduced to a formula, constitutes the genotype of the individual. However, in the case of all farm animals—unlike many plants—an individual can not reproduce by itself alone but requires the aid of a member of the opposite sex. Always the progeny of farm animals is the result of the joint contribution of two individuals, and so the progeny test must take this fact into consideration.

The situation is further complicated by the existence of two distinct groups of characters. In the one group, characters can be recognized and studied in each sex; in the second group which includes several of the most important economic characters such as milk production, litter size or egg production, the character appears in one sex alone. This state of affairs not only halves the number of progeny available for determination of the parental genotypes but renders impossible the use of the appearance or performance of the individuals of one sex as a guide to their own possible genotypes. Selection in this sex, therefore, is essentially a random process although the field of selection may be narrowed by means of sibs' appearance or performance.

Two other factors, which complicate the use of the progeny test, are of sufficient importance for mention here. One is number of progeny, the other, length of reproductive life. As regards the first, in some instances the number of offspring produced by an individual is so small as to be insufficient for satisfactory application of the progeny test. This is a serious limiting factor in the case of female sheep, horses and cattle, where the number of offspring produced during the life-time of each female is too small to provide a satisfactory random sample of her potentialities.

Length of reproductive life is another limiting factor of importance because by the time the individual has been tested and its breeding potentialities known, it is no longer capable of producing offspring. This factor will probably be found of decisive importance in deciding how to use the progeny test when dealing with such a character as rate of growth in hogs where there are difficulties in obtaining further offspring from females whose breeding potentialities have been determined.

Besides these difficulties in handling the progeny test, there is another which makes necessary further changes in applying it to farm animals. Several characters of the greatest economic importance are quantitative, each being a single expression for the activities of many genes. Present genetic technology does not permit us to analyze these characters by determining the number of genes and mode of inheritance of each, the effect of linkages and so on. We are obliged to deal with the genes in the mass as expressed in a single numerical value.

In contrast, then, with the material with which genetics ordinarily concerns itself, which is relatively simple and easy to handle, many of the most important characters, with which those who would apply genetics in the improvement of farm animals must deal, are exactly those things with which genetics finds great difficulty. Moreover, one of the tools which genetics commonly employs, crossing of races followed by inbreeding, do not yield results comparable with those obtained in the case of sim-

pler material. For one reason, domestic races of animals are not genetically pure. For a second reason, popular prejudice against so-called "racial" crosses is very great. Also, where inbreeding followed by crossing is the method employed, the expense and time required to inbreed is so great and the results of subsequent crossing so uncertain in value that progress along these lines is very doubtful. So until new methods are developed that permit of more rapid and reliable improvement than any hitherto available, reliance must be placed upon selection as the chief tool to be used in improving farm animals. Selection has as its objective the identification and propagation of those superior individuals which are believed to be capable of reproducing their good qualities in their offspring. Here the progeny test becomes extremely valuable, for by its aid—and I think that no one will question its indispensability—it is possible to obtain a reading of each breeder's worth and thus recognize those individuals which should be continued in active service as well as the best manner of mating them in the future. The progeny of the individuals thus shown most suitable for a given purpose provide a new generation of breeders to be subjected to the same rigorous tests. Moreover, if improvement in racial averages is to be made, a sufficient number of superior individuals must be discovered, and they must not only produce superior offspring on the average but they must produce them in sufficient numbers so that it becomes possible to discard all individuals of inferior breeding ability together with their progeny.

The process of selection must be continued in operation in each succeeding generation if continued improvement is desired. Here the progeny test may be used as a means for selecting in each new generation those individuals which are to be used as breeders, for among the various breeders of the last generation some have produced progeny which are superior to the progeny of others, and are, therefore, the ones from which to select parents of the next generation, which in turn are subject

to classification of breeding ability by the character of their progeny.

While all are agreed upon the indispensable nature of the progeny test, we are still confronted by the various considerations affecting its utilization which have been mentioned earlier in this paper. In some cases it is possible to follow ordinary genetic methods but, generally speaking, the progeny test, when applied to the improvement of the average value of economic characters, must be adapted to the peculiarities and requirements of each case. Improvement in yield or quality of wool may require different treatment than improvement in yield of pork and both will differ from improvement in milk yield, while the development of an all round farm horse, capable of pulling heavy loads at a rapid pace more economically than a tractor, presents still another problem. As space does not permit of a review of all possibilities, it is necessary to confine the discussion to a single instance. For this purpose the dairy cow is an excellent example because in this case the progeny test must be applied to a quantitative character, expressed by the female alone and where, moreover, it can be applied successfully only to the male because each female not only has a small number of offspring but reproduces so slowly that the few offspring obtained give just an inkling of her breeding potentialities which comes so late in her life that her further usefulness as a breeder has already passed before this small view of her breeding abilities is obtained.

Dairy production, whether it be considered as milk yield in its native state or one of its constituents, such as fat yield, is quite clearly a quantitative character resulting from the activities of many genes. No evidence has been produced that it is governed by one or two major genes. Indeed dairy production must necessarily be governed by many genes because the degree of yield is certain to be conditioned by the activities of many parts of the body other than the mammary gland.

Quantitative characters, governed by multiple genes, exhibit in inheritance peculiarities which are not exhib-

ited by the simpler forms of inheritance with which genetics commonly deals. These peculiarities not only affect the utilization of the progeny test but influence racial characteristics. As these peculiarities appear to have received less attention than their importance in dealing with quantitative characters of commercial importance warrants, I propose to consider them very briefly.

(1) The phenotype is a single numerical expression of the activities of the entire group of genes concerned with any particular quantitative character, and is the only objective criterion of their activities either singly or in the mass.

(2) There are marked differences between multiple genes according as the individual genes are non-dominant or dominant. The non-dominant will be considered first because their behavior is so simple and regular.

A. Non-dominant genes.

(a) In the case of non-dominance, a gene in the simplex condition contributes to the phenotype half of its contribution in the duplex condition. That is,—

$$AA = 2 \quad Aa = 1 \quad \text{and} \quad aa = 0$$

So also for B, C, etc.

(b) As a consequence of (a) the numerical value of the phenotype is identical with that of the genotype, and so—barring of course environmental influences—the numerical value of the breeding potentialities of any individual can be determined without resort to breeding tests, thus giving phenotypic selection of breeders a high degree of effectiveness. Consequently the usefulness of the progeny test is restricted. Space does not permit of further consideration of this point at this time.

(c) The curve of variation, when genes are non-dominant, is always symmetrical unless modified by non-genetic causes.

B. Dominant genes.

Turning now to dominant genes, a much more complicated situation is found.

(a) A gene in the simplex condition makes the same contribution to the phenotype as in the duplex. That is: AA and Aa make the same contribution to the phenotype and are therefore indistinguishable from one another. Also, they differ phenotypically from aa by the same amount. That is:

$$AA \text{ and } Aa = 2 \text{ and } aa = 0$$

(b) As a consequence of (a) the correspondence between genotype and phenotype existing in the case of non-dominant genes is lacking. The rôle of the phenotype as a basis for selection of breeders is less important than in the case of non-dominant genes and the value of the progeny test correspondingly greater.

(c) Many kinds of curves of variation result from dominant genes. Their exact character depends upon the number of genes involved, the relative contribution of each dominant gene, and the ratio of each dominant allelomorph to its recessive in the population at large. Only the briefest mention can be made of these factors and their influence upon the type of curve in relation to the utilization of the progeny test.

Suppose that each different gene contributes exactly the same amount to the phenotype, $A = B = C$, etc. Under these conditions the F_2 ratios differ from those where $A \leq B \leq C \leq D$, etc. Thus, an F_2 ratio with two pairs of genes becomes 9:6:1, instead of 9:3:3:1. Now it is convenient to work with genes of equal numerical value in deriving the theoretical consequences of dominant genes, partly because the problem is simplified but also because this value represents the central value of a series of values and so the broad outline of the curve is

established. In studying, then, the influence of number of pairs of genes upon the curve of variation, it is found that as the number of pairs increases the shape of the curve also changes gradually from a shed-roofed distribution, to a skewed curve of usual form.

The slope of this curve is governed not only by the number of different pairs of allelomorphs, but by the ratio existing in the population between each dominant allelomorph and its respective recessive. Omitting details, as the frequency of the dominant allelomorph of each pair of genes increases, the curve becomes skewed more and more toward the higher values of the phenotypes and variability tends to decrease. If the frequency of the recessive allelomorph increases, the curve of variation becomes symmetrical, flatter and of increased variability.

There is a feature of these curves which is of importance because of its bearing upon the utilization of the progeny test. When the number of genes is not too small, the genotypes corresponding to the phenotypes comprising the curve either possess the average ratio of homozygous dominants to heterozygotes or a ratio not far removed therefrom. Moreover, except when the number of pairs of allelomorphs is very few, or the ratio in the population of each dominant allelomorph to its respective recessive is large, the frequency of these phenotypes—numerically large—as well as those phenotypes that contain few or no dominants—numerically small—is very small, so small in fact that they are very unlikely to occur in random samples of the population. Ordinarily in any sample the ratio of homozygous dominants to heterozygotes will be near or at the average of the population to which they belong.

The bearing of these facts upon the determination of the breeding ability or genotype of the individual arises in this way. The characters, such as milk yield, of which a genotype or its numerical equivalent is desired are variable characters. Variability in the absence of self-

fertilization implies genetic impurity or heterozygosis. We know from the work of Hardy ('08) and others that a population maintains the same distribution of genes—and so the same degree of heterozygosis—generation after generation under conditions of random mating and equal fertility. For purposes of discussion such a population may be considered as an F_2 generation, in that the problem of applying the progeny test contains many of the same elements that one would encounter if he were working with an F_2 population of a kind where each gene could be handled separately. With such a population, one would very likely, as a first step in using the progeny test, proceed to establish a stock of multiple recessives against which to test those individuals whose breeding ability is to be determined, because he knows that the character of the progeny of such test matings will at once reveal the genotype of the individuals under test. This procedure is unquestionably the most direct means of evaluating the breeding potentialities of the individual where conditions permit of its application. Perhaps some day it may be possible to apply it in the improvement of farm animals but at present there are so many difficulties in the way of its adoption that other means must be substituted. A possible alternative is the employment of a group of average individuals against which to test breeders. But here difficulties as related to farm animals are encountered because, as in the case of dairy cattle, groups of average individuals are not as a rule practical, for the testing is done by individual farmers on herds of different levels of productive ability. A satisfactory solution of the problem of determining the breeding potentialities of the individual must take non-genetic as well as genetic factors into consideration in deciding upon the method most suitable for practical use.

Now there is a relationship between the numerical values of the parents' phenotypes and the average of the offspring's which can be utilized very satisfactorily in calculating a rating of breeding potentialities of any one

of the three when the other two are known and which, therefore, is of great practical usefulness in a case like that of the dairy sire, where the character to be improved is expressed in females alone, in that the average of the offspring tends to fall between the parents. In the case of a character wholly controlled by non-dominant genes, the offspring's average always falls exactly half-way between parents. Also, in the case of dominant genes where the ratio of recessive allelomorphs to the dominant is large so that homozygous pairs of dominant allelomorphs are few and far between while heterozygotes and homozygous recessives are numerous, the offspring's average falls at or very near the half-way point.

If, in place of non-dominant genes or dominant genes with a marked excess of recessive allelomorphs, we deal with dominant genes in which there is either equality in number between the dominant and recessive allelomorphs or an excess of dominants, the offspring no longer fall half-way between parents but tend to fall nearer the higher parent, the degree of nearness depending among other things upon the proportion of dominant allelomorphs in the population:—the greater this proportion, the nearer the offspring's average to the higher parent. The exact position of the offspring in any population of this sort will vary according to the genotypes involved in each particular mating, but the frequency distribution of the genotypes centers around an average which can be utilized very satisfactorily in obtaining a rating on the individual breeder. For example, in the case where the frequency of occurrence of each dominant allelomorph to its respective recessive is one to one, giving a phenotypic ratio of 3D to 1R for each pair of allelomorphs, the offspring's average tends to fall two thirds of the way between parents toward the higher one. This happens because throughout that portion of the phenotypic curve containing the bulk of the population, there are on the average twice as many pairs of heterozygous genes as there are homozygous dominants. As in this case only dominant genes are under consideration, a quantitative

difference between two individuals arises only when the number of pairs of homozygous recessive genes in one differs from that in the other and as, on the average, in this example there are twice as many heterozygotes as homozygous dominants in the population, it follows that the average difference is represented by three pairs of recessives in the smaller to one pair of homozygous dominants and two pairs of heterozygous dominants in the larger or some multiple of this ratio. When then two individuals are mated which differ by the average amount, it is clear that the offspring will fall two thirds of the way between parents toward the higher parent, because the mating between the homozygous dominant pair of allelomorphs and the homozygous recessive pair gives offspring which are phenotypically dominants, while the mating of the heterozygous dominants with the homozygous recessives gives offspring half of which are phenotypically dominants and half phenotypically recessives. That is, in this latter case the numerical average of the offspring's phenotypes is half-way between the parental phenotypes while in the former case the offspring's phenotype is exactly like the higher parental phenotype. Since there are twice as many of the former as the latter, the average for both kinds works out at two thirds of the difference of the numerical value of the parental phenotypes. Here is an average value for the offspring's position which can be utilized in determining either parent's phenotype or the offspring's average phenotype when any two of the three are known.

There are two features of this method of arriving at breeding potentialities which require further discussion. One is the fact that the phenotype rather than the genotype is determined. The other the fact that work is done with an average value rather than the particular value which should be known for each particular pairing of genotypes if perfect accuracy in determinating either the phenotype or genotype is to be had. These two matters are somewhat interdependent and so can not be considered entirely apart from one another.

The real question at the moment is whether by use of this method a rating of a parent can be obtained which will result in such improvement in the average of dairy cows that the business of dairying becomes profitable, not whether genetic perfection is obtained. And, as will be shown, the rating given to a parent—based upon the use of the average position of the offspring between parents—is so related to its genotype that it serves practical needs and makes possible the improvement in dairy ability which is so much needed.

The first question relates to the value of the phenotype as a guide to the breeding potentialities of the individual where multiple dominant factors are concerned. This is a numerical expression and for most individuals it bears a definite or near definite relation to the genotype because many individuals are average or near average and have the average ratio of homozygous dominants to heterozygotes, or a close approximation thereto. Of the remainder of the population there is only a small fraction where the departure from the average ratio is sufficiently great to affect the practical significance of the phenotype as a measure of its genotypic value.

As for the use of an average position of the offspring rather than that particular position which results from the particular genotypes concerned in each mating, it happens very fortunately that when the average position of the offspring is used, the calculated rating agrees with the phenotype only when matings are made between individuals in which the average ratio of homozygous dominants to heterozygotes exists. In other cases, the calculated rating is higher than the phenotype when the proportion of homozygous dominants is greater than the average and less when the proportion is less. Thus the calculated rating is parallel to the genotypes and provides a series of values which enables the breeder of farm live stock to distinguish those individuals which should be continued in service from those which should be discarded, and to mate them properly.

There is a graphic method which shows very clearly the way in which offspring fall between their parents (Fig. 1). In this method the females to which a male is

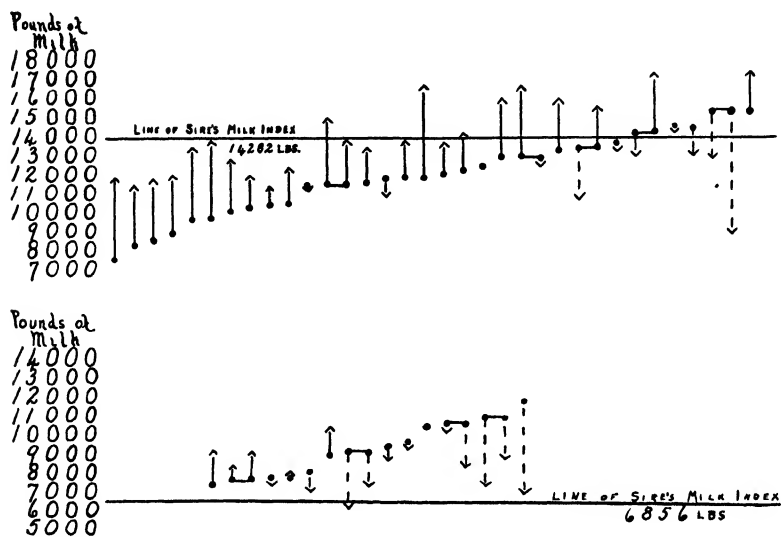


FIG. 1. A graphic method of indicating the transmitting ability of sires. Dots represent dams' character, and arrow head daughters'—in this case amount of milk—plotted against the scale at the right. Two sires are shown of which the one above is evidently superior to the one below. Charts like this can be constructed for quantitative characters in general.

mated are plotted against a scale in ascending order of their phenotypes from left to right and the offspring of each female plotted against the same scale directly above or below their respective dams. The charts of the two males show that there is a level for any particular male, below which his daughters tend to be above their dams and above which they tend to be below. This level, calculated by the method suggested above, evidently represents the individual's breeding potentialities. Of course it is possible—although not as easily accomplished—to construct charts for the female. In the case of the male, charts have been constructed for several characters in three species of animals and found to possess the same general characteristics. These charts possess special advantages for the study of the inheritance of all quantitative characters resting upon a multiple factor basis.

What use is to be made of these facts in dealing with such a case as the improvement of dairy ability, which is expressed phenotypically only in females? The absence of such expression in the males means that a guide for distinguishing dairy ability is totally lacking. Improvement is next to—if not actually—impossible when bulls are selected for breeding at random, which is what selection without a progeny test amounts to. In fact, no improvement in the inherited capacity of dairy cattle has ever been accomplished for it appears probable, from the accounts of dairy production written in the middle ages, that the dairy cattle of that time would respond to modern feed and management as well as present dairy cows. Moreover, in this case progress is hampered by the peculiarities of reproduction in dairy cattle which have been indicated earlier in the paper.

In the case of the bull, however, sufficient progeny can be obtained several years before he becomes useless as a breeder to enable a reading to be made of his potentialities as a breeder. Then the question arises as to the method to be adopted in reading the results of the progeny test. As inheritance in this instance is biparental, it is necessary in some way to determine what the progeny is receiving from their sire and what from their respective dams. If it were possible to mate the bull under test to cows that are completely homozygous for all recessive genes, or some similar uniform test material, the progeny would themselves furnish the required reading but since each of these methods is impossible of application in a practical way, the only method remaining is to take advantage of the fact that in multiple factor inheritance the offspring fall between sire and dams and to use the performance of the bull's daughters in relation to that of their dams in rating his breeding potentialities.

The rating so derived identifies both those sires that should be continued in service and is a guide to the selection of the cows to which they can be mated most advantageously. If the policy of using the right bulls on the right cows is followed continuously for a number of gen-

erations there will be a constant rise in production in the females and in the breeding potentialities of both sexes. If this plan is adopted by a group of breeders, even if the group be small, it will slowly but surely raise the average production of all dairy cattle through the distribution among farmers of the superior genotypes thus developed, especially males, of which there is always a surplus beyond the requirements of any group of breeders.

Having arrived at the conclusion that a rating on the breeding potentialities of the dairy sire can be obtained by taking advantage of the fact that his daughters' average production falls between himself and their dams, what point is to be taken as the position of the daughters to use in the calculations? Fortunately this is a matter of minor practical importance, because any position not too far from the half-way point gives ratings which are not only satisfactory in general but which possess the practical advantage of being stated in terms and amounts that are well suited for use by farmers who carry on the actual breeding operations. However, in view of the attention which has been given to this matter in the agricultural press, certain aspects of theoretical importance may be discussed briefly.

As has been shown above, the average position of the offspring relative to their parents depends upon a number of factors, but in any case there is an average position which by this fact becomes the one most suitable for use. Any other position would lie at one side of the average and would therefore be less representative of actual conditions. Nevertheless, some students of the subject believe that in all cases the offspring should be placed half-way between parents, on the ground presumably that inheritance from sire and dam is approximately equal. This is true, but genotypes are interpretations of relationships between phenotypes and are not themselves determined by observation which can be made only on phenotypes. If, then, the average numerical phenotype of the offspring is treated in every case as though it came at the half-way point, there is a departure from fact be-

cause the offspring's average numerical phenotype does, under certain conditions, fall elsewhere than half-way between parents.

Some have urged that, when the average position of the offspring's phenotype falls above the half-way point (and correspondingly for cases where it falls below), there would be an automatic increase in the quality concerned. This view appears to be based on the age-old confusion between phenotype and genotype; between appearance (or performance) and breeding ability. What really happens is that the average phenotype of the daughters is not only composed of a number of different phenotypes, but these phenotypes represent different genotypes which in turn produce, by segregation and recombination, other genotypes and their respective phenotypes, some of higher and some of lower numerical values. Thus there is a constant interchange of genes according to the rule of Hardy ('08) and so no automatic increase in the quality concerned.

Dairy cattle apparently belong in the class where the offspring's average is nearer the higher parent. Representatives of races of cattle which have different average levels of milk yield have been mated together and the position of the offspring determined. In all the published cases—ten in number—the milk yield of the resulting offspring is reported to be nearer the higher parent. The published figures indicate two thirds or thereabouts as a fair approximation to a value which can not at present be determined more accurately.

But since different races of cattle were used in these interracial matings, does not the average position of the daughters near the higher parent represent nothing more than partial heterosis? To answer this question fully would take us too far afield. All we can say here is that when hybrid vigor was the term favored for what is now called heterosis, it meant that the offspring exceeded both parents in vigor or size or some other quality. There can be no such thing as partial heterosis in the true meaning of the term. The application of the term partial

to heterosis has doubtless arisen because in discussions of the multiple factor theory it is assumed that non-dominance is the rule. In this case the offspring average exactly half-way between parents. Since in experimental crosses involving multiple factors it often happens that departures—some of them wide—from the half-way rule occur, the conclusion has been reached that heterosis is involved in some way. If the consequences of dominant genes in multiple factor inheritance had been considered, it would at once have been apparent that the departure of the offspring from the half-way point was the result of dominance associated with heterozygosis.

The case of dairy cattle illustrates the adaption of genetic principles to a particular case in bringing about improvement in a character of very large economic importance, where ordinary genetic methods are not applicable. Adaptation to circumstances is a fundamental fact of all existence, without which survival is impossible. During the last thirty-odd years a great fund of knowledge of the processes of inheritance has been accumulated by prolonged study and experiment, but only a beginning has been made in its utilization. Full utilization of genetics will be made only when the lead of physics and chemistry is followed in the use of the progeny test for, in a wide sense, the development of physics and chemistry has also been based upon a progeny test. Both have developed a wide variety of techniques for application to particular problems. Genetics must do the same. Experiments must be conducted with animals which reproduce rapidly with a view of determining the best methods of bringing about improvement in farm animals. There should be genetical engineers—who are not geneticists out of a job, but real engineers—just as there are chemical engineers.

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PROGENY TESTING IN POULTRY BREEDING AS A MEANS OF EVALUATING THE BREEDING POTENTIALITY OF AN INDIVIDUAL¹

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DURING the past 15 or 20 years in such widely separated countries as Australia, England and the United States, the progeny test has been applied by poultry breeders with considerable success in the development of high-laying strains. Although the application of the progeny test in the selection of breeding stock was limited to relatively few breeders in various countries, the success achieved in a number of cases has been quite phenomenal. Paradoxical as it may seem, during all these years the practical poultry breeder has shown no interest in the development of a formula for determining an index of the breeding potentiality of either the sire or the dam. In his application of the progeny test the practical breeder has been guided by a comparison of the laying performance of the daughters of different matings. Males and females for future breeding purposes have been selected from those families having the largest number of full-sisters with high records.

It seems to have remained for those interested in dairy breeding to first develop formulae for determining the relative breeding values of different sires. Among the formulae which have been developed, those of Yapp (1924), Goodale (1927), Warren (1932) and Wright (1932) all take into consideration the production of the dam in an attempt to evaluate the breeding potentiality of the sire. That such a method of determining the so-

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called sire's index is not applicable in the case of breeding for egg production, where the female breeders used each year have been selected on the basis of laying a minimum of 200 eggs each during their first laying year, is made clear in subsequent observations.

It is interesting to note that Warren (1932) has suggested a formula for determining not only the index of a sire mated to a number of dams but also a formula to be applied to each mating of sire and dam. For each mating the male's breeding value is estimated by adding one half of the difference between the dam's record and the average of her daughters' records to the average of the daughters' records when the daughters' average is higher than the dam's record and by subtracting one half of the difference between the dam's record and the average of her daughters' records when the daughters' average is lower than the dam's record.

Although Warren's method of determining the breeding index of a mating may result in a more nearly correct appraisal of the breeding potentialities of the male than in the case of some of the other formulae, it has been found in the case of the Rhode Island Red dams discussed in this paper that the dam's egg production is not a factor in accounting for the differences in the average egg production of the daughters of the different matings. Moreover, it is doubtful if a poultry breeder would be justified in spending the time working out a formula for the purpose of securing an index, particularly when the actual records of egg production of the daughters of various matings apparently give sufficient information for the proper appraisal of the breeding worth of a given sire and dam. That such is the case would seem to be supported by the statement of Edwards (1932), who has emphasized the weaknesses of various sires' indices used in dairy breeding and has suggested that the average yield of the daughters of a bull is the most satisfactory indicator of a bull's transmitting ability.

In order to determine the manner in which progeny testing in poultry breeding can be used in evaluating the breeding potentiality of either sire or dam, the results secured from each of a considerable number of matings made at the U. S. Animal Husbandry Experiment Farm, Beltsville, Maryland, are presented here for discussion. The results include those secured from various Rhode Island Red males used during the breeding seasons of 1928, 1929 and 1930. Males producing relatively few breeding daughters or families of less than three full-sisters per family have been omitted; otherwise all the daughters of each dam were placed in the laying houses except those physically unfit and in rare cases a pullet with a serious standard-bred defect or a disqualification. The records of egg production for the years 1928-1929, 1929-1930 and 1930-1931 were chosen as a basis of discussion for the reason that during these laying years all daughters of various matings that were placed in the laying houses were kept throughout their first year of laying except birds which died. The first laying year in the case of each bird embraces 365 days from the date she laid her first egg.

There were 1,013 Rhode Island Red daughters produced during the three years. Mortality amounted to 9.18 per cent. and the 920 daughters that completed their first year of laying averaged 192.03 eggs each.

The environmental conditions under which the daughters were kept during the three years were as identical as it was possible to maintain. The hatching season each year occurred from March 17 to the first week in May. Housing conditions were identical, the rations fed and the methods of feeding were the same, and the general methods of management were the same. Artificial lights were used in the morning during the winter months.

Table I gives the number of the sire, the number of dams to which he was mated, the average egg production of the dams, the number of daughters produced and the average egg production of the daughters. The data in

Table I involve 19 different sires, 128 dams and 729 daughters. Each sire was from a dam that laid 200 or more eggs during her first laying year. Each dam laid 200 or more eggs during her first laying year, the dams' average production, given in Table I, being based upon first-year egg production, the dams having been bred for the first time as yearlings, although several were used in their third and fourth years.

TABLE I

THE MEAN EGG PRODUCTION OF THE DAMS MATED TO VARIOUS SIRES AND
THE AVERAGE EGG PRODUCTION OF THEIR DAUGHTERS

Year	Sire No.	No. of dams	Dams' mean production	No. of daughters	Daughters' production
1928	173	8	222.75	48	219.41
"	200	6	243.00	52	192.64
"	10105	9	241.70	49	215.83
"	10102	4	207.50	16	180.25
"	10101	4	215.15	20	197.65
"	10104	5	232.00	26	198.54
1929	325	4	239.00	17	186.59
"	326	3	244.00	11	171.36
"	330	7	246.43	46	200.63
"	334	7	231.29	37	210.78
"	337	2	208.00	11	141.64
"	338	7	238.00	31	206.35
"	351	11	232.27	75	170.84
"	352	7	231.71	37	196.86
1930	330	7	250.14	38	195.21
"	334	6	241.67	32	205.16
"	338	11	250.36	60	192.35
"	368	10	230.20	58	204.55
"	363	7	239.00	44	196.61
"	369	3	252.67	21	179.48

The question might well be raised as to the validity of comparing the results of the mating of a given sire with the same or different dam or a given dam with the same or different sire in each of two years. Concerning the Rhode Island Reds discussed in this paper, it may be said that comparisons of the results secured from various matings made in the three years involved appear to be

perfectly valid. It will be recalled that the daughters produced each year were given as nearly identical environmental conditions as possible from year to year. The average egg production per bird in each of the three years was as follows:

1928	195.41
1929	184.13
1930	197.87

Although it has been determined statistically that the 1929 egg production is significantly lower than that of either 1928 or 1930, the relatively low average in 1929 is apparently chiefly due to the use of sires 326, 337 and 351, three sires that proved to be among the poorest breeders of all Rhode Island Red sires used during the three years. Moreover, the average egg production per bird in White Leghorns in each of the years 1928, 1929 and 1930 was practically the same, *viz.*: 192.17, 193.50 and 193.84, respectively.

In the breeding of various classes of live stock it has frequently been suggested that the dam's record of production is indicative of her breeding potentiality. Warren (1932) has said that "the dam's inherited tendencies for egg production are indicated by her individual egg record; but the male's tendencies are unknown." Concerning this suggestion, it is probably true that among the fowls of the universe the domestic biddy of to-day with a 200-egg record has greater potentialities for transmitting good egg production to her progeny than has a wild fowl of the bamboo jungles of India that lays about 15 eggs and then goes broody. However, in a selected group of females, selected on the basis of having laid 200 or more eggs during the first year of laying, the dam's record of egg production apparently has little or no significance, as shown by the data in Table II.

In order to eliminate the influence of the male in determining whether or not the dam's record of egg production bears any relation to the mean egg production of the dam's daughters, data from 13 pens of Rhode Island

Reds with a sufficient number of matings per pen are available. In Table II, for each pen, the mean of the mean egg production of the daughters of each dam whose egg production was *above* the mean egg production per dam for the pen is compared with the mean of the mean egg production of the daughters of each dam whose egg production was *below* the mean egg production per dam for the pen.

Since it has been urged from time to time that a female having a number of sisters with high records is more apt to transmit high egg production to her daughters than a female having a number of sisters with low records, the data have been analyzed to determine whether or not such might be the case with the selected Rhode Island Red dams under review. In Table II, for each pen, the mean of the mean egg production of the daughters of each dam whose egg production together with that of her full-sisters was *above* the mean of the mean egg production of the full-sisters of all dams in each pen is compared with the mean of the mean egg production of the daughters of each dam whose egg production, together with that of her full-sisters, was *below* the mean of the mean egg production of the full-sisters of all dams in each pen.

From the standpoint of the relationship between the record of egg production of the dam and the mean egg production of her daughters, the data in the third and fourth columns of Table II show that there was relatively little difference between the egg production of the daughters whose dam's record was *above* the mean egg production per dam for each pen and the egg production of the daughters whose dam's record was *below* the mean egg production per dam for each pen. In 6 pens out of the 13 the dams whose records were *above* the dams' mean for the pen produced daughters whose mean egg production was lower than the mean egg production of the daughters produced by dams whose records were *below* the dams' mean for the pen; in the thirteenth pen the

TABLE II

THE MEAN OF THE MEAN EGG PRODUCTION OF THE DAUGHTERS OF RHODE ISLAND RED DAMS IN EACH OF 13 PENS TREATED FROM THE STANDPOINT OF ELIMINATING THE INFLUENCE OF THE MALE IN DETERMINING THE INFLUENCE OF THE DAM'S RECORD OF EGG PRODUCTION AND IN DETERMINING THE INFLUENCE OF THE MEAN EGG PRODUCTION OF THE DAM'S FULL-SISTER FAMILY IN TRANSMITTING EGG-LAYING ABILITY

Sire	Year	The mean of the mean egg production of daughters whose dams' records were—		The mean of the mean egg production of daughters whose dams' full-sister families' records were—	
		Above the mean egg production per dam for the pen*	Below the mean egg production per dam for the pen*	Above the mean of the egg production of the full-sisters*	Below the mean of the egg production of the full-sisters*
173	1928	215	222	206	226
200	"	189	184	196	190
10105	"	214	193	204	211
338	1929	205	204	207	202
330	"	202	203	200	206
352	"	190	204	193	203
334	"	202	218	213	208
351	"	175	169	161	176
363	1930	215	175	198	197
338	"	184	199	198	175
330	"	199	194	194	198
334	"	204	215	193	216
368	"	205	205	198	207

* Decimals have been omitted.

daughters of the dams whose records were *above* the dams' mean for the pen laid the same number of eggs as the daughters of the dams whose records were *below* the dams' mean for the pen. Therefore, in this selected group of Rhode Island Red dams mated to selected sires the record of the dam does not serve as an index of her ability to transmit egg production to her daughters.

The data in the last two columns of Table II also indicate that the mean egg production of a group of full-sisters, of which the dam is one, does not serve as an index of the dam's breeding potentiality.

The correlation between the egg production of the dams and the egg production of their daughters is 0.04

± 0.06 and the correlation between the average egg production of full-sisters of the dams and the average egg production of the daughters of the dams is 0.04 ± 0.06 , neither correlation being significant.

The results secured from matings in which the same sire was mated to two different groups of dams two different years show that the average egg production of the daughters of each of the two groups of daughters was fairly uniform. Sires Nos. 330, 334 and 338 were each used in 1929 and 1930, the average egg production of the daughters produced each year being as follows (taken from Table I):

Sire No.	1929	1930
330	200.63	195.21
334	210.78	205.16
338	206.35	192.35

The difference between the mean egg production of the daughters produced in 1929 and 1930 in the case of each sire is not significant, from which it follows that the two groups of dams mated to the same sire did not differ on the average in their breeding potentialities.

TABLE III
THE MEAN EGG PRODUCTION OF DAUGHTERS OF DAMS EACH MATED TO TWO DIFFERENT SIRES

Dam No.	Dam's production	First year			Second year		
		Sire No.	No. of daughters	Daughters' mean production*	Sire No.	No. of daughters	Daughters' mean production*
5829	246	325	4	189	338	4	241
6266	251	325	5	189	338	3	188
6112	243	200	9	194	352	3	202
6238	245	200	14	223	352	6	201
6414	242	200	5	179	352	5	191
6142	242	351	9	182	368	9	220
6312	230	351	8	192	368	5	210
6407	224	351	7	194	368	8	210
6428	210	351	7	178	368	6	169

* Decimals have been omitted.

The results secured from matings in which the same dam was mated to two different sires two different years are interesting and are given in Table III.

The results given in Table III show that in some cases the daughters of a dam mated to one male have a higher mean egg production than the daughters of the same dam mated to another male, whereas another dam mated to the same two males may give opposite results.

The results secured when the same sire is mated to the same dam two years in succession show that although in most cases the mean egg production of the two groups of daughters is quite comparable, there are a few cases in which the differences are rather great. The results secured from such matings are given in Table IV.

TABLE IV

FOR EACH OF TWO YEARS THE MEAN EGG PRODUCTION OF DAUGHTERS OF THE SAME DAM MATED TO THE SAME SIRE

Sire No.	Dam No.	Dam's production	First year		Second year	
			No. of daughters	Daughters' mean production*	No. of daughters	Daughters' mean production*
173	5416	229	8	228	8	243
173	5551	214	6	213	5	205
176	6024	212	8	217	5	189
176	6027	206	6	178	4	222
330	6213	271	7	177	5	198
330	6217	254	7	220	4	192
334	6401	233	6	214	3	184
338	6462	260	6	215	7	217

* Decimals have been omitted.

If the results given in Tables III and IV are compared, it is observed that the differences between the mean egg production of the daughters produced each year by matings of the same sire \times same dam are practically as large as are the differences between the mean egg production of the daughters produced each year by matings of different sire \times same dam.

The data in Table IV show that since the average egg production of the daughters secured from the mating of a given sire and a given dam one year may differ considerably from the average egg production of the daughters secured from the same mating another year, the possibility of establishing a reliable index representing the breeding potentiality of either the sire or the dam seems problematical. This would seem to be especially true in the breeding of some of the larger classes of live-stock because of the relatively small number of progeny produced by most sires, to say nothing of the limited number of progeny produced by most dams. Then again, there apparently have been very few cases in which large numbers of progeny from various matings have been kept under uniform environmental conditions, as was the case with the Rhode Island Red daughters whose records are under discussion.

That full-brothers may produce significantly different results concerning the mean egg production of their daughters is shown by the results given in Table V.

TABLE V
THE MEAN EGG PRODUCTION OF DAUGHTERS OF TWO FULL-BROTHERS

Sire No.	No. of dams	Dam's mean production	No. of daughters	Daughters' mean production
200	6	243.00	52	192.64 \pm 3.72
10105	9	241.70	49	215.83 \pm 5.05
Difference				23.19 \pm 6.27

Although the difference in mean egg production between the two groups of daughters in Table V is significant, it is interesting to note that the mating of sire 200 \times dam 6238 produced fourteen daughters whose mean egg production was 223.00 eggs, which is slightly above the mean of all daughters of 10105. It is to be noted further that the mean egg production of the daughters of sire 200 \times dam 6238 exceeded the mean egg production of the daughters of six out of the nine dams mated to sire 10105.

The poultry breeder is interested not only in the mean egg production of all the daughters of each of all sires used but also in the mean egg production of the daughters of each dam. It is quite possible that the mean egg production of the daughters of a given dam may be of greater importance in the selection of future breeding stock than the mean egg production of all the daughters of the same sire to which the given dam was mated. In other words, the poultry breeder is vitally interested in the results secured from each individual mating.

The mean egg production of daughters of each two or more full-sisters mated to a different sire sometimes differ quite markedly, as shown in Table VI.

TABLE VI
THE MEAN EGG PRODUCTION OF DAUGHTERS OF EACH OF TWO FULL-SISTERS
MATED TO A DIFFERENT SIRE

Dam No.*	Dam's production	Sire No.	No. of daughters	Daughters' mean production†
5677	220	173†	4	186
5679	261	10105	3	206
5932	211	10105†	4	215
5967	226	10101	5	179
6213	271	330	7	177
6303	238	338†	7	209
6142	242	351	10	182
6218	230	334†	6	220
6144	223	200	13	178
6254	227	334†	6	215
6436	250	325	3	178
6537	245	363†	9	211
6668	271	330	8	207
6695	247	368†	3	174

* Full-sisters are shown in pairs.

† Indicates the superior sire of each pair in respect to the mean egg production of all daughters produced, as given in Table I.

‡ Decimals have been omitted.

From the standpoint of the results secured from each pair of sires mated to full-sisters, the data in Table VI indicate that in 5 cases out of 7 in which the mean egg

production of the daughters of one sire was higher, the mean egg production of all the daughters of that sire mated to various dams was also higher, as shown in Table I.

Quite different results are sometimes secured from matings of a sire to full-sisters, as shown in Table VII. The dam's egg production has been omitted in order to save space.

TABLE VII

THE MEAN EGG PRODUCTION OF DAUGHTERS OF EACH OF TWO OR MORE FULL-SISTERS MATED TO THE SAME SIRE

Sire No.	Dam No.*	No. of daughters	Daughters' mean production†	Sire No.	Dam No.*	No. of daughters	Daughters' mean production†
173	5829	8	209	338	6502	7	188
	5846	7	201		6627	4	203
	5928	5	207		6630	5	192
330	6200	10	208	363	6752	6	169
	6213	7	177		6773	6	191
330	6465	3	231		6792	6	204
	6532	6	180	363	6537	9	211
	6668	8	207		6577	4	178
330	6549	7	205		6735	3	166
	6844	5	159		6750	7	221
351	6312	8	192	368	6312	5	210
	6428	7	178		6428	6	169
352	6264	6	169	10105	5680	3	127
	6400	7	208		5923	4	214

* Dams mated to the same sire are full-sisters.

† Decimals have been omitted.

The data given in Table VII show that in some cases the mean egg production of the daughters of full-sisters mated to the same sire varied as much as 30 or more eggs. It is apparent that full-sisters selected on the basis of having laid a minimum of 200 eggs each may differ quite markedly in their breeding potentialities, irrespective of

the actual number of eggs they laid during their first year of laying. Theoretically, the logical way of determining the relative breeding potentialities of a group of full-sisters or any other group of dams would be to mate such dams to males that have the genes for egg production in a recessive condition. The difficulty of identifying males of this kind is obvious to any one who has made a study of the inheritance of such a complex character as egg-laying ability. If such were possible it might be further possible to establish the fact that the egg production, within a certain range, of selected dams is significantly correlated with breeding potentiality.

That sires may differ in their breeding potentialities is indicated by the differences in the mean egg production of their daughters, as given in Table I. The mean egg production of the daughters of the sires given in the table varied from 141.64 to 219.40, although in a number of cases the differences in the mean egg production are not significant, as, for instance, the daughters of sire No. 10101 with a mean egg production of 197.65 and the daughters of sire No. 10104 with a mean egg production of 198.54. It is true that the number of daughters of certain sires is too small to attach much significance to their mean egg production. However, in order to determine whether or not the difference between the mean egg production of the daughters of certain sires is significant, the results secured from three sires with relatively large

TABLE VIII

THE SIGNIFICANCE OF THE DIFFERENCE IN THE MEAN EGG PRODUCTION OF THE DAUGHTERS OF EACH OF THREE SIRES

Sire No.	No. of daughters	Daughters' mean production	Sire No.	No. of daughters	Daughters' mean production	Difference
173	48	219.41 \pm 4.74	351	75	170.84 \pm 3.58	48.57 \pm 5.94
173	48	219.41 \pm 4.74	338	60	192.35 \pm 3.90	27.06 \pm 6.14
338	60	192.35 \pm 3.90	351	75	170.84 \pm 3.58	21.51 \pm 5.29

families have been analyzed and the data are presented in Table VIII.

The data in the last column of Table VIII show that the difference in the mean egg production between the daughters of each pair of sires is significant, indicating that the three sires differ among themselves concerning their breeding potentialities. A proper appraisal of the breeding potentiality of each sire would be possible if he could be mated to dams having the same genes for egg production in a recessive condition.

In conclusion, the results secured from various matings of Rhode Island Red sires, whose dams laid a minimum of 200 eggs each during their first laying year, and dams which laid a minimum of 200 eggs each during their first laying year seem to justify the following observations:

(1) The number of eggs laid by the dam could not be used as a criterion of her breeding potentialities.

(2) Therefore, in the case of this selected group of dams, their first-year records of egg production should not be used in the determination of a sire's breeding index.

(3) The mean egg production of full-sisters could not be used as an index of the breeding potentialities of any of the full-sisters.

(4) At the same time, various dams, even full-sisters, may apparently differ among themselves in their breeding potentialities.

(5) Also full-brothers may differ among themselves in their breeding potentialities.

(6) Since certain sires have been shown to differ among themselves in their breeding potentialities, it follows that the mean egg production of each sire's daughters serves as the most readily available index of a given sire's breeding potentiality.

(7) In addition, the mean egg production of the daughters of the mating of a given sire and a given dam is the index of greatest value in determining the breeding potentiality of an individual sire or dam.

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THE PROGENY TEST AND METHODS OF BREEDING APPROPRIATE TO CERTAIN SPECIES OF CROP PLANTS¹

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REPEATED attempts by plant breeders, working with different species of both self-fertilized and cross-fertilized crops, have failed to discover any sound basis for selection of parent plants except that which involves a progeny test. With self-fertilized crops the progeny test may be directly applied because such plants reproduce with fidelity and there is no appreciable loss of vigor from one generation to the next. In the cross-fertilized crops, however, the question of how to utilize the progeny test to best advantage has not been clarified by investigation and it is somewhat complicated by fundamental differences in the breeding behavior of species as well as the nature of problems which confront the breeder of these crops. Everything considered, we believe that although our method of dealing with this topic may be somewhat indirect, most good will come from a general discussion of breeding methods in relation to species and objectives and by confining our attention to the normally cross-fertilized crops. We will then consider briefly some aspects of the progeny test in its practical application.

Let us consider first the improvement of herbage plants in general and certain legumes and grasses in particular. Discussion will be facilitated by selecting a typical problem from among the many which are likely to engage the attention of plant breeders in their efforts to improve these crops. Perhaps no more important objective could be chosen than that of developing a superior strain for grazing purposes.

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Species of herbage plants are sometimes grown in pure cultures but more often in simple or complex mixtures. In order to secure good establishment and a dense sward for pasture purposes, the seed is sown thickly. This results in intensive competition between species and even between plants of the same species. Dominance of one species over another is profoundly influenced by climatic, edaphic and biotic factors. Growth can be influenced differentially by the application of fertilizers and methods of grazing, but unless a strain is well adapted to its environment, it will fail to hold its own or compete successfully with inferior volunteer species and weedy plants.

To say that a variety or strain possesses a high degree of adaptation for pasture purposes implies that it possesses the necessary physiological and morphological characteristics which fit it for a particular environment. The requirements are exacting and frequently complex. In addition to good yield under favorable conditions there may be included drought resistance, winter hardiness, longevity or resistance to certain diseases. To these may be added those characters which directly affect its utility, as, for example, a satisfactory nutrient content and palatability, and early or late maturity. There must also be a nice balance between production of herbage and yield of seed, since one tends to develop at the expense of the other.

Having considered in a general way the nature of the problem involved in producing a new variety for pasture purposes, we are now in a position to state more precisely what are the essential qualities which such a strain should possess. First and foremost it must possess the greatest possible range of adaptation so that its usefulness will not be too greatly restricted; secondly, it must be composed of plants of the required type for pasture purposes; and finally it must be as productive as possible of high quality herbage, consistent with satisfactory seeding habits.

What methods are available to the plant breeder for developing these improved strains and what procedure is likely to be most direct and effective? These may be con-

sidered conveniently under three headings as follows: (1) selfed line breeding, (2) strain building, (3) mass selection.

SELFED LINE BREEDING

The method of breeding cross-fertilized plants which involves selection within selfed lines and subsequent hybridization has been widely applied in corn breeding. It is generally recognized by plant breeders as a genetically sound basis for corn improvement. Experimental evidence seems to show that it offers the best opportunity for obtaining substantial improvement in the yield of this crop. Richey (8) and others have reviewed the literature and presented comprehensive discussions covering all phases of the subject.

It must be recognized, however, that selfed line breeding has distinct limitations when applied to most other cross-fertilized crops. In many respects the corn crop lends itself particularly well to inbreeding and the utilization of selfed lines. Its mode of reproduction makes it possible to produce selfed seed and hybrid seed with a minimum of difficulty and expense. With respect to ease of manipulation it is far superior to any other of the cross-fertilized crops. The physical difficulties involved in selfing large numbers of plants in most of the herbage species is a problem that must be recognized, and the same applies to the production of hybrid seed.

While single and double crosses between selfed lines have materially increased the production of corn, it is worthy of note that the synthesizing of several selfed lines to form a new strain has not been equally productive of increased yields. This has also been found to be true in the case of rye. Evidently a general increase in yields as a result of selfed line breeding will have to come through the use of hybrid seed produced annually for field planting. This practise would be out of the question with any of the species of herbage plants. Selfed lines in these crops of necessity would have to be utilized in the form of synthetic varieties.

Inbreeding results in loss of vigor in nearly all species of herbage plants that are normally cross-fertilized. This applies to alfalfa, red clover, timothy, orchard grass, perennial rye grass, and many others. Since it does not appear that it will ever be possible to capitalize on hybrid vigor in these species through the use of F_1 seed, and since selfing is accompanied by pronounced loss in vigor of growth, the question arises as to how far this loss in vigor can be recovered in synthetic strains produced by controlled mating or random crossing of selfed lines.

Even if experience should show that maximum vigor can be restored and maintained under experimental conditions by selfed line breeding, it is altogether likely that other factors will be lost in the process, factors which may influence profoundly the physiological response of the strain to the natural environment. Where natural adaptation and ability to compete with other species is of first consideration, loss of some of the essential physiological factors which characterized the parent material should be a matter of serious concern. It is quite conceivable that the best way to secure strains of herbage plants which are capable of standing up under the varied conditions to which they will be subjected is to bring together as wide a range of selected genotypes as possible. Experience has shown that improved strains of grasses which trace their origin to single plant selections have proved disappointing when tested in places remote from those in which they originated. On the other hand, there are a considerable number of very useful strains which have originated through a process of natural selection, as, for example, Grimm alfalfa in America, Ultuna red clover in Sweden, Akaora cocksfoot and Hawke's Bay perennial rye grass in New Zealand, and Kentish wild white clover in England.

STRAIN BUILDING

The term "strain building" was first coined by Jenkin (2) to describe the methods which he employed in breed-

ing perennial rye grass. It was used by him in a very broad sense to include any system of mating by which a strain is built up by crossing from carefully selected plants. We will use the term in this sense also and as such it is to be distinguished from "Selfed line breeding," which presupposes several generations of inbreeding until the parent plants are reasonably homozygous. At the same time "strain building" does not exclude inbreeding entirely such as occurs, for example, in backcrosses. In other words, it may be thought of as comparable to live-stock breeding where foundation stock is selected on type and performance both of the parents and their progenies.

The method of "strain building" involves the composite crossing of few or many carefully selected parent plants. The number of plants contributing to the new strain will depend to a considerable extent on the number of unrelated individuals available which possess the necessary qualifications, and this in turn will depend in practice on the nature of the problem. If the objective is primarily the production of a persistent leafy pasture strain, as in the present case, one has only to select for growth form and productivity within the population of an appropriate habitat, in which case there is likely to be no lack of parent material. On the other hand, if it is desired to breed for some special attribute which is of comparatively rare occurrence in the species, it will be necessary to choose only those plants as parents which possess the character or characters in question. This may restrict the basis of the strain to a very few plants. Under these circumstances some degree of inbreeding is inevitable. It may even be advisable to practise repeated selfing in order to secure homozygosity as quickly as possible but it should be recognized that this will be done at the expense of vigor of growth and possibly other factors of economic importance. Very frequently, also, inbreeding results in partial or complete self-sterility, in which case out-crossing is the only alternative.

"Strain building" may be regarded as a breeding procedure directed essentially toward concentrating certain morphological or physiological characters in a particular strain. It does not necessarily contemplate complete uniformity but aims rather to increase the proportion of individuals in the population that possess the desired characteristics. The ideal, of course, is complete homozygosity but as Stapledon (9) has suggested, "the economic breeder of herbage plants may hope to achieve valuable results without necessarily becoming a slave to the idea of genetical purity."

As outlined above, the method is very broad in its application. It admits of different systems of mating and various methods of selecting parent plants. It aims to maintain vigor, as far as this is consistent with progress toward the desired result, by bringing into the strain as wide a range of genotypes as possible. The method, however, presupposes the conscious selection of parent plants as foundation stock and systematic selection within families as a basis for further crossing. In evaluating the breeding potentialities of the parent plants there can be no better guide than that obtained from a study of the progenies which they produce when mated with other individuals.

MASS SELECTION

Studies by Turesson (11) and others have shown conclusively that there exist within the species definite hereditary habitat types and that the habitat type represents the genotypical response of the species-population to a particular set of environmental conditions. They have also shown that the individuals which compose these habitat types, or "ecotypes" as they are called by Turesson, tend to be phenotypically uniform but genetically more or less heterogeneous, depending on the stability of the habitat. Climate plays the leading rôle as a modifying agent, but other factors also exert a very great influence on the character of the population, such as soil type and the effect produced by close grazing. There is

ample justification for the contention that mass selection properly employed offers a ready means of capitalizing on the work which nature has accomplished through natural selection over long periods of time. This is particularly true in the case of grasses and legumes and especially those which are required for pasture purposes. Long-continued grazing of old pastures has been shown to operate as a modifying agent to produce a race of growth-forms especially suited to a pasture habitat. It is to be expected that such a habitat will provide the best possible source of foundation plants with which to build an improved pasture strain. There is reason to believe also that when mass selection methods are adopted, the larger the number of suitable parent plants that can be brought into the strain the better, and that special methods should be adopted to insure the greatest possible degree of random-pollination.

SELFED-LINE BREEDING VS. STRAIN BUILDING

The method of breeding which one chooses to adopt with any particular species will be dictated very largely by the breeding behavior of the species with respect to fertility relationships and the degree to which the plants are reduced in vigor by selfing. In this connection it may be profitable to consider very briefly these two factors in a few of the major crop plants and how they are likely to influence the choice of appropriate breeding methods.

Alfalfa: Alfalfa plants, for the most part, are cross-fertilized, but they are also, to a considerable extent, self-fertilized. The total quantity of seed produced and the relative amount of selfing under field conditions is profoundly influenced by weather conditions at the time of pollination. Alfalfa is moderately self-fertile when artificially self-pollinated. Inbreeding is accompanied by a very marked reduction in self-fertility which is out of all proportion to the very considerable decrease in vegetative growth.

The writer (5, 6) has conducted experiments with alfalfa for the past ten years, primarily to determine the

effects of selfed line breeding and its possibilities as a practical method of improvement. The most conspicuous result of inbreeding was seen to be a marked reduction in vigor and seed production. This could be observed up to and including the fourth generation.

The results of selfed line breeding with alfalfa in these experiments have not been impressive as a practical method of improvement. In addition to the physical difficulty of handling more than a few score selfed lines, there is the problem of greatly reduced fertility. This amounted to 70 per cent. in the second generation, and in the fourth, almost complete self-sterility in a large proportion of the families. Williams (15) found that the average yield of seed from first generation selfed lines was only 12.4 per cent. of that of the parent plants from which they were derived.

Not only is self-fertility greatly reduced by inbreeding, but cross-fertility also appears to be much less. Our results show that where 65 fourth generation selfed lines were grown together in a compact field under conditions favorable for cross-pollination, with the standard variety in every fourth row, the average seed yield of the inbred families was only 22 per cent. of the standard variety. Vigor of growth, on the other hand, as measured by total yield of plants, was reduced to only 54 per cent. This emphasizes the marked effect of inbreeding on fertility.

We are now attempting to develop seed production from another angle which appears quite promising. Five unrelated mother plants from a highly adapted variety are available which produce a normal crop of seed without artificial manipulation or the visitation of insects. One of these plants has matured five crops of seed under a variety of conditions (field and greenhouse) and a full quota of pods have always been secured. This family has been inbred to the third generation with consequent loss in vigor. Seed production has been so seriously impaired that it has become necessary to abandon selfing and resort to out-crossing with the other plants. By inter-crossing and back-crossing

it should be possible to attain homozygosity of the factors involved without much loss in vigor, providing the character is inherited in a simple manner. Otherwise a larger number of suitable mother plants will be necessary.

The difficulties which have been mentioned when selfed line breeding is adopted with alfalfa suggests that "strain building" is likely to be the better method. This conclusion is illustrated beautifully by the problem stated above in connection with developing a strain of alfalfa which is highly self-fertile and also self-pollinating. It is typical of a large class of problems that may be encountered in the breeding of cross-fertilized crops. Among these may be mentioned the breeding of a disease-resistant strain, when resistant parent plants are of rare occurrence. On the other hand it is not so difficult to produce strains which reproduce themselves with comparative purity for specialized growth-forms because plants of a given type are usually fairly abundant, in which case it is far more important to adopt a method of breeding which will maintain maximum vigor than to attain genetical purity.

Red clover: Self-sterility in red clover is a physiological phenomenon due to the inhibiting action of the stylar tissue. Williams (14) concludes that this species, for all practical purposes, can be regarded as being self-sterile. This view appears to be amply justified by the facts. He states also that loss of vigor resulting from inbreeding presents even greater difficulties in the improvement of red clover than cross-incompatibility. The reduction was found to be very pronounced in the second generation, becoming less at each subsequent crossing until the fourth or fifth generation was reached, after which there was no apparent loss. The average loss in vigor in the first to the third generation was given as 50 to 60 per cent. Inbreeding, however, except in so far as it decreased the chances of cross-fertilization, had no direct effect on fertility or on the size and quality of the seed. It would seem from this that red clover is dis-

tinctly different from alfalfa with respect to the effect of inbreeding on seed production.

Red clover appears to be an example of a very important species of cross-fertilized crop which can best be improved by composite crossing. Of necessity, a method of breeding must be adopted which aims to utilize parent plants which are qualified to contribute particular genetic factors, where genetic purity is required, or those which are phenotypically similar where the chief consideration is ecotypic homogeneity of the strain aggregate.

Perennial grasses: It is unnecessary for our purpose to discuss at any great length the breeding behavior of the different cultivated grasses, except to survey very briefly the behavior of a few important economic species in relation to fertility and vegetative vigor under conditions of selfing and crossing.

Valle (12) investigated the tall Finnish type of timothy with reference to the effects of inbreeding and concluded that the selfing method can not lead to practical results. He was unable to obtain any constant self-fertile lines, and inbreeding resulted in loss of vigor. Self-sterility was found to increase progressively with continued selfing. Jenkin (4) studied self-fertility in fifty plants of the semi-hay type of timothy. Forty-three of these were of very low fertility, yielding less than one seed per centimeter of inflorescence. On the other hand, a few plants were very highly self-fertile. These results agree in general with those of other workers.

In a study of over 1,200 plants of orchard grass, *Dactylis glomerata*, which were selfed, Stapledon (10) found that the degree of self-fertility varied from absolute self-fertility to apparent complete self-fertility. On the average, plants of orchard grass were found to be about half as vigorous when derived from selfing as when obtained by hybridization.

The results of fertility studies in perennial rye grass, *Lolium perenne*, have been presented by Jenkin (33). These emphasize the low degree of self-fertility in this species. It was estimated that 50 per cent. of the plants

set less than 1 per cent. of the possible number of seeds, while 75 per cent. of the plants set less than 3 per cent. Only in two plants per hundred was seed setting greater than 20 per cent., or one seed per spikelet. These results are in general agreement with those reported by Gregor (1). With respect to loss in vigor due to selfing, Jenkin reports that reduction in yield is well over 40 per cent. on the average as compared with that produced by intercrossing.

The writer has conducted breeding experiments with awnless brome grass, *Bromus inermis*, for the past ten years in which 100 selfed lines were grown in each of four generations. Self-sterility has increased in each generation and much loss in vigor has taken place. This decrease in yield was not very apparent until the third generation, but in the fourth it became very pronounced.

The findings which we have summarized from the work of several investigators on four important economic grasses emphasize how wide-spread are the deleterious effects of inbreeding in cross-fertilized crops. These in turn have a very important bearing on breeding procedure, as has been pointed out in connection with the other species previously discussed.

Potatoes: There are some interesting features of the potato crop and potato breeding which are worth mentioning in connection with the relative value of "selfed line breeding" and "strain breeding" methods. Although the potato plant is normally self-fertilized, the cultivated strains are extremely heterozygous, due to the fact that they originated as hybrids and are propagated from tubers. With respect to ease of self- and cross-pollination and mode of propagation, the potato is especially well suited to selfed line breeding because all the hybrid vigor which results from the crossing of superior selfed lines can be retained, since segregation in subsequent generations is avoided by vegetative propagation. Loss in vigor due to inbreeding, although very pronounced in the potato, is not in itself a serious drawback, for reasons already given. Pollen sterility, on the other

hand, presents a difficulty of some magnitude, since it is almost complete in the major economic varieties. The problem of securing viable pollen, however, is probably at least as great, whether the method adopted be out-crossing between unrelated plants or systematic inbreeding. Everything considered, therefore, on theoretical grounds, it might be expected that the method of "selection within self-fertilized lines" should find its most ideal application in the breeding of potatoes. This view is strongly supported by Krantz (7).

There is one angle to the question, however, which remains an unknown quantity as far as success in the breeding of economic varieties of potatoes is concerned, namely, the effect which continuous inbreeding may have by restricting the genetic constitutions of the plants and thus narrowing their range of adaptation. Since many factors of physiological significance probably can not be measured in terms of hybrid vigor, it is reasonable to suppose that some of them may be lost through inbreeding. On these grounds we are justified in awaiting the verdict of conclusive experimental evidence to show that strains of potatoes, and other cross-fertilized crops, produced by the method of selfed line breeding, possess the necessary stamina to meet the rough and tumble of practical farm conditions. It is well known that many otherwise admirable varieties of crop plants have not measured up to this test, and it is at least debatable whether their failure may not have been due to a too much restricted inheritance. On the other hand, it is not difficult to name many varieties of crop plants, including potatoes, with a remarkably wide range of adaptation, which originated either from very wide crosses or composite crossing.

Having pointed out that selfed line breeding is especially applicable to the potato crop and having suggested a possible limitation of that method of improvement, we can now go further and say that the potato plant should provide exceptionally good material for studying the

differential effects of inbreeding as between vigor and other physiological qualities of practical importance. Because of the fact that maximum hybrid vigor can be retained by vegetative propagation, a conspicuous lack of adaptive response to differences of environment, beyond that which would normally occur with standard sorts, may logically be attributed to inbreeding.

THE PROGENY TEST

Having considered methods of breeding in relation to the practical improvement of cross-fertilized crop plants, a few observations may be added with reference to the progeny test in its practical application.

It may be taken for granted that the progeny test in one form or another provides the only method of evaluating the breeding potentialities of an individual. The progeny may be produced from selfed seed or from hybrid seed of crosses between homozygous or heterozygous individuals. With all these three classes of parent material the progeny test is extensively employed, but the information obtained in each case differs greatly in significance and precision.

When the progeny test is conducted with selfed seed of heterozygous plants, it is easy to measure the relative vigor of growth, but this information, if obtained in the actively segregating generations, is of very little value. This was clearly demonstrated in experiments by the writer with both alfalfa and brome grass. It was found that very pronounced loss in vigor may occur in one of the first four or five generations of inbreeding. A striking example may be cited to illustrate this point. One "original" parent plant of alfalfa produced a very vigorous first generation progeny and four excellent second generation selfed lines. The latter were regarded as the best out of about 80 progenies in this project. These were again self-fertilized, but all the third generation selfed lines were almost too weak to survive, although sixteen of them were grown.

Results such as these were not infrequent, but nevertheless high yielding first generation progenies, on the average, tended to produce the most vigorous selfed lines in succeeding generations. Exceptions, however, were so numerous as to make the progeny test ineffective when vigor of growth was taken as a basis of selection.

With respect to phenotypic selection, our experience has been that progeny tests can be used to considerable advantage. In general, the first generation selfed lines of alfalfa gave a fair indication of growth-form and also the extent to which parent plants will breed true for a particular type. Although segregation takes place for many morphological characters in nearly all selfed lines, successive generations tend to exhibit the same general characteristics in a fair proportion of families. It is possible, therefore, to eliminate a considerable number of "original" parent plants on the basis of general appearance in first and second generation selfed lines.

The second class of progeny tests referred to above is that in which hybrid seed from homozygous parents is used, as in the case of single crosses between selfed lines of corn. In this case where the parents are required for the production of F_1 seed, the progeny test provides a direct method of evaluating the breeding potentialities and all other characters of economic importance. F_1 hybrids provide also the best indication that we have of the value of parent plants in composite crossing, but for this purpose such progeny tests are not necessarily reliable.

The third class of progeny tests is that in which heterozygous parent plants, when inter-crossed, are evaluated on the performance of their progenies. Such a procedure is necessary in the breeding of red clover, since here cross-fertilization is practically obligatory. In this case the plants are extremely heterozygous. Williams (13) successfully employs the progeny test with this species. Carefully selected plants of similar type are paired and mated in various combinations. Those which consistently produce high yielding and otherwise desirable progenies when out-crossed with other individuals are

labeled as good parents. This is the method employed by animal breeders. There seems no good reason why it should not be practised by plant breeders wherever selfed line breeding may be found impossible or undesirable.

One thing in particular relating to the progeny test deserves special emphasis. This is the supreme importance of conducting such tests under environmental conditions similar to those in which the variety presumably will be grown. Plant breeding must be initiated at certain centers but it is a great advantage if promising strains in the making can be subjected to the incidence of extremes of temperature, drought, disease organisms or whatever factors may influence the success of the new production. Attempts have been made to simulate nature by providing artificial conditions in the laboratory for conducting progeny tests, and these have been moderately successful in certain cases, but generally speaking few tests have yet been devised which approximate natural conditions. More rapid progress in this direction would greatly facilitate the work of the plant breeders.

SUMMARY

Methods of procedure in breeding self-fertilized species of crop plants have been fairly well standardized. The same can not be said with reference to cross-fertilized crops. The latter include such widely different species as corn, alfalfa, red clover, timothy and many other grasses and legumes. These vary greatly in their mode of reproduction and breeding behavior. Improvement of these species presents a variety of problems, depending on how they are to be cultured and utilized. Methods of breeding should be used, which are appropriate to the species concerned and the objective to be attained.

Breeding methods commonly employed can be grouped under three headings—selfed line breeding, strain building and mass selection. These are discussed and compared with respect to their applicability to different species and specific problems.

"Selfed line breeding" has been employed most extensively in corn improvement. Although apparently well adapted to this crop, it has only a limited usefulness in the breeding of herbage plants.

"Strain building" involves the crossing of few or many carefully selected parent plants. It admits of different systems of mating and aims to maintain vigor of growth by bringing into the strain as wide a range of genotypes as possible, as far as this is consistent with progress toward the desired result. Inbreeding may be detrimental in so far as it tends to narrow the lines of inheritance and restrict the range of adaptation of the strain.

In nature there exists within the species definite hereditary habitat types which represent the genotypical response of the species-population to a particular habitat. "Mass selection" provides a ready means of capitalizing on the work which nature has accomplished.

The progeny test provides the only reliable method of evaluating the breeding potentialities of parent plants. The information to be gained from such tests differs greatly in significance and precision depending on whether the progeny is grown from selfed seed or from hybrid seed of crosses between homozygous or heterozygous individuals. It is of supreme importance that progenies shall be subjected to the incidence of those environmental factors which presumably may influence the success of the new production.

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PROGRESSIVE MUTATIONS INDUCED IN *GOSSYPIUM HIRSUTUM* BY RADIATIONS¹

W. R. HORLACHER AND D. T. KILLOUGH²

GENE mutations in both directions at a single locus have been reported by Patterson and Muller (1930), Hanson (1928), and Timofeeff-Ressovsky (1929a, 1929b, 1931) as having been produced in *Drosophila* by radiations. Stadler (1931) has noted similar phenomena in maize. These cases are important because of the evidence they present that recessive mutations are not losses of genes and because they show that mutations which are beneficial to the organism, progressive mutations, can arise.

Two progressive mutations have been induced by radiations in cotton (*Gossypium hirsutum*). The evidence that these mutations had occurred in the somatic tissues of cotton plants grown from x-rayed seeds was presented in a recent paper (Horlacher and Killough, 1931). Breeding evidence has now been secured to show that both of these mutations are transmitted to succeeding generations.

The first of the progressive mutations to be discussed is the one from forked leaf shape to normal leaf shape. Seeds containing embryos known to be heterozygous for leaf shape were x-rayed and plants grown from these. The bolls from these plants were selfed and the individual boll progeny grown separately. Normal segregation for leaf shape gives 1 normal leaf: 2 intermediate leaf: 1 forked leaf. A mutation from forked to normal in a branch of the heterozygous plant (Nn to NN) would produce

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a boll from which all the progeny would have normal leaf shape. Likewise a mutation from normal to forked (Nn to nn) would produce a boll from which all the progeny would have forked leaves. A total of 529 individual boll progenies were grown. Among these a number of bolls produced only normal leaf progeny and a few produced only forked leaf progeny. These results are shown in detail in Table 1.

TABLE 1

No. of bolls	Plants from each boll			Odds against this being a chance deviation from the 1: 2: 1 ratio
	Normal leaf	Interme- diate leaf	Forked leaf	
2	8	0	0	65,535: 1
1	5	0	0	1,023: 1
4	4	0	0	255: 1
4	3	0	0	63: 1
10	2	0	0	15: 1
28	1	0	0	3: 1
1	0	0	3	63: 1
6	0	0	2	15: 1
15	0	0	1	3: 1

The two bolls having 8 normal leaf plants each are very highly indicative of a mutation from n to N in each case. The other bolls furnish supporting evidence. Selfed bolls from these normal leaf plants have been found to produce 100 per cent. normal leaf progeny. This proves that these plants are homozygous. Of the 49 bolls listed in Table 1 as producing only normal leaf plants, 8 were from plant 1-59. This plant had one other boll which gave 9 normal leaf and 7 intermediate leaf progeny. Five more of the bolls producing only normal leaf progeny were from plant 1-50. This plant had 9 other bolls, which segregated for the different leaf shapes. These two plants produced all the bolls which gave 4 or more progeny all of which had normal leaf shape. They both furnish good evidence that a mutation has been produced from forked leaf to normal leaf shape.

Selfed seed from these normal leaf plants have produced all normal leaf progeny. None of the 22 bolls listed in Table 1 as producing only forked leaf progeny produced a large enough number of offspring to give good evidence that a mutation had been produced from normal leaf to forked leaf. They are included here, however, as giving some indication of the possibility of producing mutations in both directions at this locus.

There are two other possible explanations of the production of the bolls giving 100 per cent. normal leaf progeny by the x-ray treatment of the heterozygous seeds. The first is that the radiation has caused a deletion of the chromosome carrying the gene n for forked leaf. This would leave the plant monosomic for the chromosome carrying the gene N for normal leaf. In that case all the functional gametes would probably carry the chromosome with N and would produce only normal leaf progeny. The same reasoning would apply in case there was a deletion of only that portion of the chromosome carrying the gene n .

The other possible explanation of this variation is that a gametic lethal which is closely linked with n has been induced. The genetic constitution of the plant under this condition would be $(NL)(nl)$, l representing the gametic lethal. The only functional gametes produced would be NL . The union of two of these, $(NL)(NL)$, would produce normal leaf progeny and that would be the only type of progeny produced by a boll coming from such tissue.

Evidence for the production of a mutation from forked leaf to normal leaf has also been secured from another source. This case does not lend itself to interpretation by either of the two alternative hypotheses suggested above because the gene N for normal leaf was not in this stock before it was irradiated. Plant No. 30-41, grown from an x-rayed seed of homozygous forked leaf cotton, produced 6 bolls, 5 of which gave forked and intermediate leaf progeny, while the other boll (B5) gave 1 normal

and 2 intermediate leaf progeny. The intermediate leaf progeny may represent in some cases variants within the homozygous forked genotype, but in other cases they may be the result of gene changes. The normal leaf progeny, however, could have resulted only from a mutation, as even an accidental cross pollination of a forked leaf plant by a normal leaf plant would result in the production of intermediate leaf shape. There are, thus, three cases of induced mutations of the forked leaf gene to its allelomorph, the normal leaf gene. These constitute mutations in fewer than one per cent. of the seeds x-rayed.

The normal leaf has much more leaf surface than does the forked leaf. In the normal leaf the lobes are broad and the indentations between the lobes are not very deep. The lobes of the forked leaf are very narrow, almost pencil-like, and the indentations are very deep. The normal leaf, therefore, provides the plant with more food-making surface than does the forked leaf. Considered on this basis, the mutation from forked leaf to normal leaf is progressive.

The other progressive mutation is the one from virescent yellow leaf and plant color to normal green. Virescent yellow cotton does not have as much chlorophyll as does normal green cotton. The yellow pigment which is normally present in all cotton plants shows along with the reduced amount of chlorophyll which is found in these plants giving the characteristic virescent yellow color to these plants. This cotton does not grow as tall as normal green cotton, neither does it yield as much lint cotton per plant.

The virescent yellow mutant character was obtained from R. F. Karper and R. F. Dickson, who discovered two plants of this type in a large field of Mebane cotton on the Experiment Station at Spur, Texas, in 1925. Only two plants with this characteristic appeared in this field. Virescent yellow was a type hitherto unknown and it appears likely that these virescent yellow plants were the result of a mutation. Subsequent tests by Killough

(1929) showed these two plants to be homozygous virescent yellow (*vv*) and a simple recessive to green. He reported a ratio of 545 green to 177 virescent yellow in the segregating generation.

Several hundred seeds of virescent yellow cotton were x-rayed. A considerable percentage of the plants grown from these seeds had green areas of greater or less extent. From self-fertilized flowers on some of the plants with green areas 66 progeny were grown. Of these, 13 were normal green plants. These 13 green plants came from four different bolls. The fact that these green plants developed from self-fertilized bolls of virescent yellow cotton grown from x-rayed seed is good evidence that they resulted from induced mutation and not from cross pollination. In order to secure further proof that these green plants were due to induced mutations and not to accidental cross pollinations, flowers on these plants were selfed and progeny grown therefrom. If cross pollination had occurred all of the green plants would of necessity be heterozygous and the progeny would segregate in the ratio of 3 green to 1 virescent yellow. If the green plants were due to mutations, however, an occasional homozygous green plant might be expected in this group, although the majority of the green plants resulting from induced mutations would be heterozygous. The occurrence of a homozygous green plant would be definite proof of an induced mutation. One of the 13 green plants produced 21 green and no virescent yellow progeny. This plant was undoubtedly homozygous green, as the odds against the chance production of 21 dominants and no recessives by a heterozygous plant are 420:1. The other 12 of these 13 plants proved to be heterozygous.

Proof that a mutation has been induced by radiations from virescent yellow to green is shown by the facts that (1) 13 green plants arose from self-fertilized bolls of irradiated virescent yellow cotton, (2) one of these 13 green plants was homozygous green, and (3) green

plants have never arisen from virescent yellow under any other conditions.

The mutation rate from virescent yellow to green was less than one per cent.

The green plants resulting from the induced mutations from virescent yellow to green were larger, thriftier and more vigorous than the virescent yellow plants from the same bolls. It is advantageous to the plant to have the amount of chlorophyll that is present in the normal green plant rather than the amount that is in virescent yellow cotton. This mutation may, therefore, also be considered as progressive.

Another important consideration regarding this mutation is that it is the reversal of a mutation that had previously occurred in nature. Virescent yellow, as pointed out above, undoubtedly arose as a natural mutation from green. This gene has later mutated back to green under the influence of radiations.

SUMMARY

Mutations which are progressive have been produced in cotton (*Gossypium hirsutum*) by x-ray treatment of the dry seeds. These mutations consist of:

1. A mutation from forked leaf shape to normal leaf shape. This was induced in two different lines of cotton, viz.,

a. An F_1 which was heterozygous, Nn .

b. A homozygous forked leaf line, nn .

2. A mutation from virescent yellow leaf and plant color to normal green leaf and plant color.

The mutation rate in each case was less than one per cent.

Evidence is also presented for reversible mutations in cotton. There are indications that a mutation has been induced from normal leaf to forked leaf, the reverse of the mutation from forked leaf to normal leaf. The induced mutation from virescent yellow to green is the

reversal of the mutation from green to virescent yellow which had previously occurred in nature.

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CROSSING PRODUCTION AND EXHIBITION RHODE ISLAND REDS¹

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EXHIBITION-BRED Rhode Island Red chicks were hatched and reared with pedigreed production-bred chicks in the spring of 1929. The former group was hatched from eggs furnished by one of the leading breeders of the world. The pedigreed chicks had an ancestry that had been selected and bred for high fecundity at the Massachusetts Agricultural Experiment Station since 1913. The two groups of chicks differed widely in plumage color, to some extent in rate of growth and to a marked degree in age at which sexual maturity was attained. Crosses were made in 1930 between these two types and this report concerns itself with some of the results in F_1 and F_2 with respect to characteristics affecting annual egg production.

Goodale and Sanborn (1922) pointed out that the number of eggs laid in a year depends very largely upon age at sexual maturity, winter pause, high intensity, broodiness and persistency. Hays and Sanborn (1927) have shown the net relation of each of these five traits to annual egg record. Hays (1924) reports the mode of inheritance of the first four and Hays (1927) reports on the inheritance of persistency. In general, the highest producer must carry these five desirable inherited traits and the true-breeding high producer will have the following genetic formula: $E_eE'EmmIII'T'aaccPP$.

SEXUAL MATURITY

The approach of sexual maturity in the individual is definitely recognizable in the female by the laying of eggs. In the male no specific criterion of sexual maturity is recognized. There are, however, certain external

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changes which suggest that the male is sexually-mature and may reproduce. Such secondary sexual characters as the adult male type of comb, the male type of adult plumage and the beginning of spur development probably indicate complete sexual maturity. But these secondary characters are relative only and as such can not be measured in relation to the appearance of functional spermatozoa in the male reproductive system. This report will, therefore, deal only with female descendants.

Early sexual maturity has been shown to be associated with high annual egg production by Rice (1915), Hurst (1921), Kempster (1922, 1925, 1926 and 1927), Ball and Alder (1917), Kennard (1921), Hays, Sanborn and James (1924), Jull (1923 and 1924), Parkhurst and Buster (1927), Knox (1930) and others. Hays and Sanborn (1927b, *loc. cit.*) showed, however, that early sexual maturity as such did not affect annual egg production but that early maturity affects production because it is associated with high intensity and very intimately associated with high persistency. Hays (1927) pointed out the linkage between gene E' for early maturity and gene P for high persistency.

TABLE 1
SEXUAL MATURITY

Exhibition Sire J 711 (Eee'e')	Production Dams (Early)			
	J 1683 (EoE'e')	J 2247 (EoE'e')	J 2662 (Eoe'e')	J 2666 (EoE'e')
F ₁ Daughters				
36 Early				
18 Late				
F ₂ Daughters				
18 Early				
22 Late				
3 Early				
13 Late				

Phenotypically early maturing pullets begin laying at 215 days or younger, late maturing at 216 days or older.

P₁ dams were all phenotypically early and, mated to an exhibition male, gave 36 early and 18 late daughters.

Early maturing F_1 dams mated to F_1 males (two used) gave 18 early and 22 late.

Late maturing F_1 dams mated to one F_1 male gave 3 early and 13 late.

These data indicate that the recessive condition $e'e'$ was present in the exhibition line.

INTENSITY

Dryden (1921) used the two highest months in the yearly record as a measure of intensity and was able to make progress by selecting breeders on this basis. Hurst (1921, *loc. cit.*) employed the rate of laying as a percentage basis throughout the first year to measure intensity—30 per cent. being considered high. Goodale and Sanborn (1922) suggest as measures of intensity the months, the initial cycle, the inter-broody periods and the spring period. Harris and Goodale (1922) used the monthly basis as a measure of intensity as has been done by others. Hays and Sanborn (1927a) studied four possible measures and decided that mean winter clutch size is the most dependable.

Concerning the mode of inheritance of intensity, Hurst (1921, *loc. cit.*) reports that high winter intensity and high spring intensity each depend upon distinct dominant genes (W. S.). He believes that high autumn intensity depends upon a recessive (m).

Hays and Sanborn (1927a, *loc. cit.*) state that a mean winter clutch size of three or more eggs depends upon the presence of two dominant genes I and I', that a clutch size of more than two but less than three depends upon gene I and that a clutch size of two or less indicates that both genes I and I' are absent.

In these studies the females are grouped into three phenotypes with respect to winter clutch size, namely, II', Ii', and ii for clutch averages of 3+, 2.1 to 2.9 and 2-, respectively.

P_1 hens were all II' in that their clutch size was three or more and, mated to an exhibition male, gave 27 II', 18

TABLE 2
INTENSITY

Exhibition Sire J 711 (iiI'i')	Production Dams (All Intense)			
	J 1683 (II'I')	J 2247 (II'I')	J 2662 (II'I')	J 2666 (II'I')
F ₁ Daughters				
27 II'				
18 Ii'				
9 ii'				
F ₂ Daughters				
5 II'				
16 Ii'				
20 ii'				
1 II'				
3 Ii'				
6 ii'				

Ii', and 9 ii' daughters in F₁. This ratio shows that the male lacked one dominant gene for high intensity and that the females were not homozygous for both genes.

Highly intense (II') F₁ females mated to an F₁ male gave 5 II', 16 Ii' and 20 ii daughters, again indicating the low intensity from the grandsire.

Two low intensity F₁ hens mated to an F₁ male gave 1 II', 3 Ii' and 6 ii' daughters in F₂.

WINTER PAUSE

The cessation of production before March first of the pullet year has been called winter pause by Goodale and Sanborn (1922, *loc. cit.*). Hays (1924, *loc. cit.*) pointed out that winter pause behaves as a simple dominant in inheritance. Hays and Sanborn (1926) arbitrarily adopted a four-day cessation of production before March as indicating pause. Hays and Sanborn (1927, *loc. cit.*) showed that the duration of winter pause produced a very significant reduction in annual egg yield.

Birds pausing for four or more days are designated M while those lacking such a pause are m.

Three P₁ dams lacking pause (mm) mated to an exhibition male gave F₁ daughters, 25 non-pause to 11 pause.

One P₁ dam with pause gave 9 m to 9 M daughters.

TABLE 3
WINTER PAUSE

Exhibition Sire J 711 (Mm)	Production Dams* = Pause			
	J 1683 (mm)	J 2247* (Mm)	J 2662 (mm)	J 2666 (mm)
<u>F₁ Daughters</u>				
25 Non-pause		9 Non-pause		
11 Pause		9 Pause		
<u>F₂ Daughters</u>				
19 Non-pause		3 Non-pause		
19 Pause		10 Pause		

Mating four non-pause F₁ females to an F₁ male gave 19 m to 19 M F₂ daughters.

Mating two pause F₁ females to an F₁ male gave 3 non-pause to 10 pause F₂ daughters.

These data clearly indicate an inherited tendency to pause.

BROODINESS

Bateson (1902) and Hurst (1905) present data on crosses between broody and non-broody races indicating the dominant nature of the broody instinct. Hurst (1913) reports that broodiness behaves as a simple dominant. Goodale, Sanborn and White (1920) suggested that three genes might be concerned in the inheritance of broodiness. Punnett and Bailey (1920) partially confirmed Goodale's observations. Hays (1924, *loc. cit.*) confirmed the observation of Goodale that broodiness depends in its inheritance upon two dominant genes A and C, but was unable to discover a dominant inhibitor N.

Hays and Sanborn (1926a) showed that broody birds lay significantly fewer eggs than do non-broody birds. Hays and Sanborn (1927, *loc. cit.*) presented further conclusive evidence on the effect of duration of broodiness on egg yield.

Females are roughly designated AC if they go broody and ac if non-broody in the pullet year.

TABLE 4
BROODINESS

Exhibition Sire J 711 (AaCc)	Production Dams* = Broody		
	J 1683 (Aacc)	J 2247* (AaCc)	J 2666* (AaCC)

F₁ Daughters

6 Non-broody	10 Non-broody
2 Broody	19 Broody

F₂ Daughters

One non-broody P₁ hen was mated to an exhibition male giving 6 non-broody and 2 broody F₁ daughters.

Two broody P₁ hens mated to the above male gave 10 non-broody to 19 broody F₁ daughters.

These data suggest that at least one of the broody genes was carried by the exhibition male. The F₂ daughters are now in their first laying year.

PERSISTENCY

Persistency followed by late molting at the end of the pullet laying year has long been recognized as a desirable characteristic associated with high egg records. Rice (1915, *loc. cit.*) stressed the importance of high persistency. Harris, *et al.* (1917, 1918, 1921, 1922), showed an important relation between fall laying and annual record. Goodale and Sanborn (1922, *loc. cit.*) consider persistency as a major, desirable characteristic. Hays and Sanborn (1927, *loc. cit.*) showed that persistency is the most important of the five traits considered in relation to egg records.

Hurst (1921, *loc. cit.*) states that high persistency is inherited as a simple Mendelian recessive. Hays (1927, *loc. cit.*) found high persistency to be transmitted as a simple dominant in Rhode Island Reds.

Birds laying for 315 days or more are persistent.

Three P₁ females, all high in persistency, were mated to an exhibition male.

TABLE 5
PERSISTENCY

Exhibition Sire J 711 (Pp)	Production Dams (All Persistent)		
	J 1683 (Pp)	J 2247 (Pp)	J 2666 (Pp)

F₁ Daughters

14 Persistent
23 Non persistent

F₂ Daughters

The F₁ daughters showed 14 persistent to 23 non-persistent. This is an excess of non-persistent birds over the expectation of equality. No explanation is offered until further data are studied. Records on F₂ daughters are not yet complete.

ANNUAL PRODUCTION

The three P₁ production-bred females with complete records had annual egg records as follows: 261, 224, 224. These, when mated to an exhibition male, gave a total of 37 F₁ daughters. These F₁ daughters from the three dams gave the following mean records: 175, 190 and 209. The outstanding trait accountable for the superior record of the last family of daughters was persistency.

EGG SIZE

The mean egg size of most of the birds was recorded. Each egg was weighed during the period from February 22 to March 15, inclusive. Data reported (Hays, 1930) and other data on the flock indicate that first year maximum egg size appears either in February or March for the flock as a whole. This fact would suggest that maximum egg weight is reached in the pullet year at about ten months of age, which probably indicates, according to Waters' (1931) studies, that mature body weight is attained at the same time.

Birds may be arbitrarily classed as large-egg individuals if their eggs average 56.7 grams or more in Febru-

ary and March. Small-egg birds would lay eggs below this weight.

Of the four production-bred P_1 females, three laid large eggs and one laid small eggs. The F_1 generation of daughters showed 21 large to 12 small. The F_2 daughters were 18 large to 8 small. Egg size is superior in the exhibition strain and the F_2 generation shows a higher proportion of large-egg birds than the F_1 .

TABLE 6
GENOTYPES

P_1 Sire
J 711—Eee'e'iiI'i'MmAaCcPp
P_1 Dams
J 1683—EoE'e'IiI'i'mmAaccPp
J 2247—EoE'e'IiI'i'MaAaCcPp
J 2662—Eoe'e'IiI'i'mm-----
J 2666—EoE,e'IiI'i'mmAaCCPp

SUMMARY

In general, these data indicate that early sexual maturity behaves as a dominant in crosses between early-maturing production-bred and late-maturing exhibition-bred Rhode Island Reds. High intensity behaves in the same general way as early sexual maturity. Winter pause behaves as a dominant trait. Broodiness was increased by crossing, probably due to the introduction of one of the essential complementary genes from each strain. There is an excess of non-persistent F_1 daughters over expectation on the basis of simple dominance. Annual egg records were smaller in F_1 daughters than in the production strain. Egg weight at mature body weight increased in F_2 over F_1 .

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AN ALLEGED CASE OF INHERITANCE OF ACQUIRED CHARACTERS

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ON May 4, 1932, I received the following letter from the librarian of the City Library, Springfield, Massachusetts:

A young married woman on my staff, when about twelve years of age, sustained an injury to her hand by being hit with a croquet mallet, which resulted in the disappearance of the knuckle, a marked shortening of the finger, and a slight curvature of the finger. She tells me that her daughter, about ten years of age, shows an almost identical deformity. This would appear to be a case of the inheritance of an acquired character.

She wrote about the matter to a university professor in Texas, who replied that such a result was impossible. This seemed to be a strange attitude for a scientist. I have understood that rare cases of the apparent inheritance of an acquired character have been reported, and I should suppose that a scientist would be interested to verify the facts rather than to declare them impossible on general principles.

On May 14 the following letter was received from Mrs. Ernestine R. Fernald,* of the Springfield Library:

Mr. Wellman has asked me to reply to your letter of May 12, addressed to him, as I and my daughter are the subjects of the discussion in question, and perhaps can supply more complete information.

First, I will describe the finger as it is now—of necessity, in untechnical language. The knuckle bone, which ordinarily humps up in closing the fist, is gone entirely, and this is the most important bit of evidence I have to prove that my daughter has inherited a mutilation, as I will explain further on. The finger appears to be one-half inch shorter than the corresponding finger on the other hand but actually it is not, because the joint (where it joins the hand) is now about a half inch further into the hand (toward the wrist) than normal, leaving the actual finger-bone of normal length. [See Fig. 1.]

The accident occurred as follows: I reached for my croquet ball to pick it up and move it, and my friend at the same moment started to knock it over to me with her mallet. Her mallet came down with much force on that knuckle. Immediately after the accident the *knuckle-bone was there* and humped up as usual but pushed back from its usual position. This I know is positively true. I remember seeing it so clearly and after it was set that night (I took ether) there was much talk between the doctor and my mother over the fact that the little knuckle bone had dropped down in some

* Permission granted to print name.

place but that I was young enough so that it would undoubtedly be absorbed—as it has been.

My daughter's hand is identical with mine, as it is now Not almost the same, but identical as far as can be observed from the outside (See Fig 1)

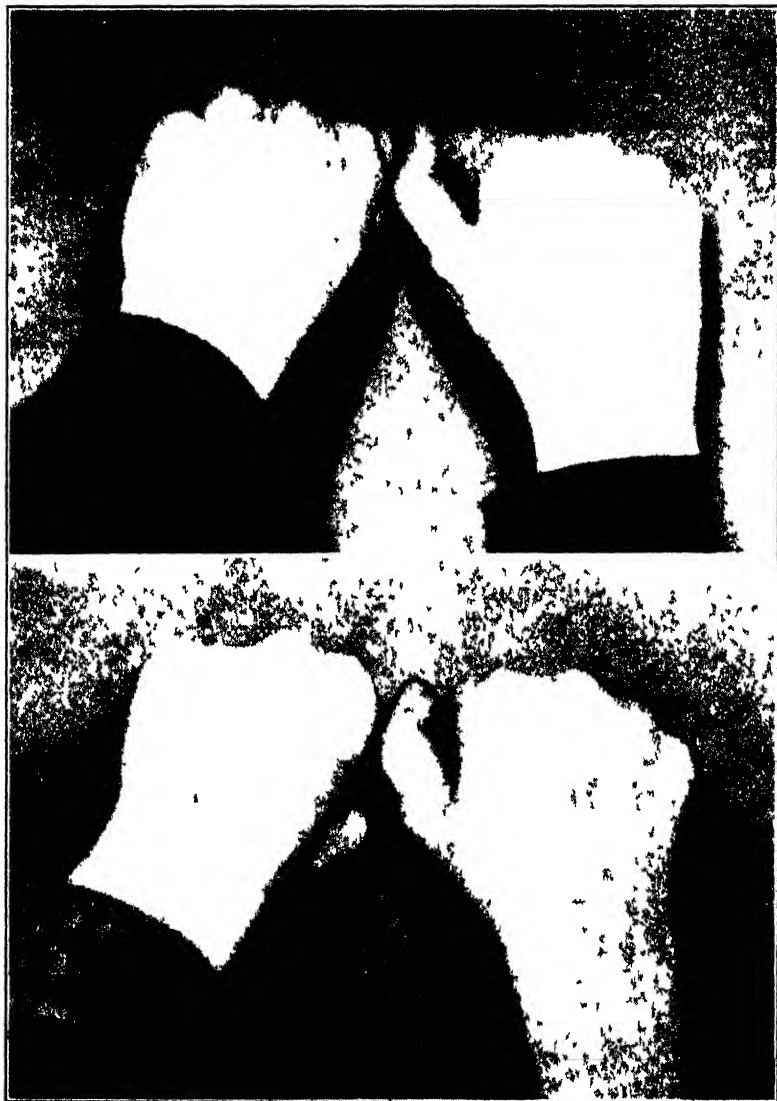


FIG. 1. Photograph of the hands of Mrs. Fernald, showing short fourth metacarpal on left hand (above); and of Elizabeth Fernald, showing short fourth metacarpal on left hand (below).

-In view of the above facts, it would not matter much whether there was any malformation of that finger—and I am very sure there was not—before the accident or not, would it? At least it was definitely different after the accident, and my daughter's is exactly as mine is now.

Perhaps this is sufficient explanation for this letter. If you wish any more facts or details, I will be glad to supply them. My interest in the matter has been whetted by the many books I have read which say it can not happen, when I know it has happened!

X-rays of the hand of Mrs. Fernald and her daughter, Elizabeth, showed that in both cases the fourth metacarpal of the left hand was shorter than the other metacarpals, about 28 per cent. less than the average of the third and fifth metacarpals (Figs. 1-2). In the daughter of 10½ years the fourth metacarpal shows no such separate epiphysis as is found at the distal end of the second, third and fifth metacarpals. If a short epiphysis was ever formed it was of small size and fused early with the diathesis. In the mother the shaft of the bone is slenderer in all the metacarpals than in the daughter. The distal end of the fourth metacarpal is similarly molded to that of the daughter.

Mrs. Fernald having stated that she was interested in genealogy the suggestion was made to her to request all her relatives to send in outlines of their hands and feet, and similar outlines were obtained from Mrs. Fernald, Elizabeth Fernald and Mrs. Fernald's father.

Since Mrs. Fernald's mother is dead, it was impossible to make first-hand observations upon her hand. Her father's hand, however, showed no deformity; only a slight extension of the web between second and third toes. An examination of the contours of hand and feet sent in by Mrs. Fernald showed no other case of an especially short finger, although in five cases out of eighteen in the family the fourth digit is shorter than the second by a few (1 to 8) millimeters. The persons showing this near equality of the fourth and second digits are in two cases on Mrs. Fernald's father's side (first cousins of Mrs. Fernald) and in one case on her mother's side. No abnormalities of the toes of the feet were found in the



FIG. 2. Radiograph of left hand of Mrs. Fernald, showing short fourth metacarpal bone (left); and of left hand of Elizabeth Fernald, showing short fourth metacarpal bone (right).



FIG. 3. Radiograph of hands of Ada B., showing short fourth metacarpal bone on right hand (a); the third and fifth metacarpals also reduced in length. On the left hand (b) the fifth metacarpal is greatly reduced in length, the third and fourth metacarpals slightly reduced.

propositus or collaterals. Correspondence brought out, however, two things: One, that a first cousin once removed of Mrs. Fernald stated that Mrs. Fernald's mother's father had short fingers perfectly formed. Also it was learned that a second cousin of Elizabeth Fernald by the name of Ada B. (III, 1), living in Detroit, had a short metacarpal on the right hand. X-rays were kindly furnished by this woman, and are shown in Fig. 3. These reveal a short fifth (also fourth) metacarpal on the left hand and a short fourth (also fifth) metacarpal on the right hand. This metacarpal of the right hand is very different in form from that of Mrs. Fernald, the axis being somewhat curved. Miss B.'s mother's fourth digit on left hand is 6 mm shorter than the second digit; while the two are equal on the right hand. Also, her right fifth finger is exceptionally short.

The pedigree chart of the family of the mother's side of Mrs. Fernald showing affected individuals is given in Fig. 4.

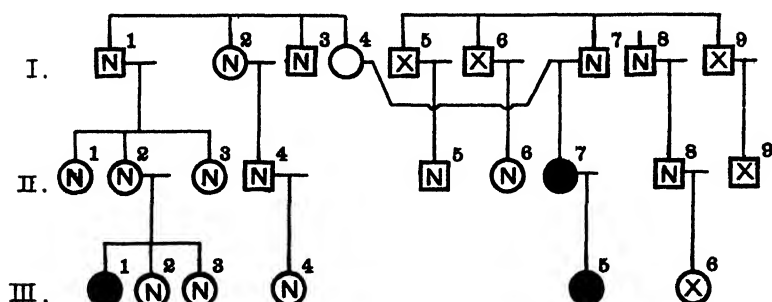


FIG. 4. Pedigree chart of Fernald-Morse-B. family. Squares, males; circles, females. Black symbols, persons with strikingly short metacarpals. N, hands that do not deviate strikingly from the average hand. X, no data. The father of generation I 1-4 (not charted) is said to have had short fingers, as did his brother and this brother's daughter. I 7 has a slight webbing, zygodactyly, between 2nd and 3rd toes, seen also in his daughter II 7 and her daughter III 5, also in his brother and another brother's son. Such a slight zygodactyly is found in a considerable fraction (say 5 per cent.) of the population. II 2 has on the left hand (only) a fourth digit that is 6 mm shorter than the second. On the right hand the fifth digit is exceptionally short. II 4 has a slight webbing between 2nd and 3rd toes on both feet. II 5 hands and feet of average form. III 4 has a slight zygodactyly between 2nd and 3rd toes of right foot (only).

An examination of the files of the Eugenics Record Office reveals a somewhat similar case in a young woman who furnished photographs, as shown in Fig. 5. In this

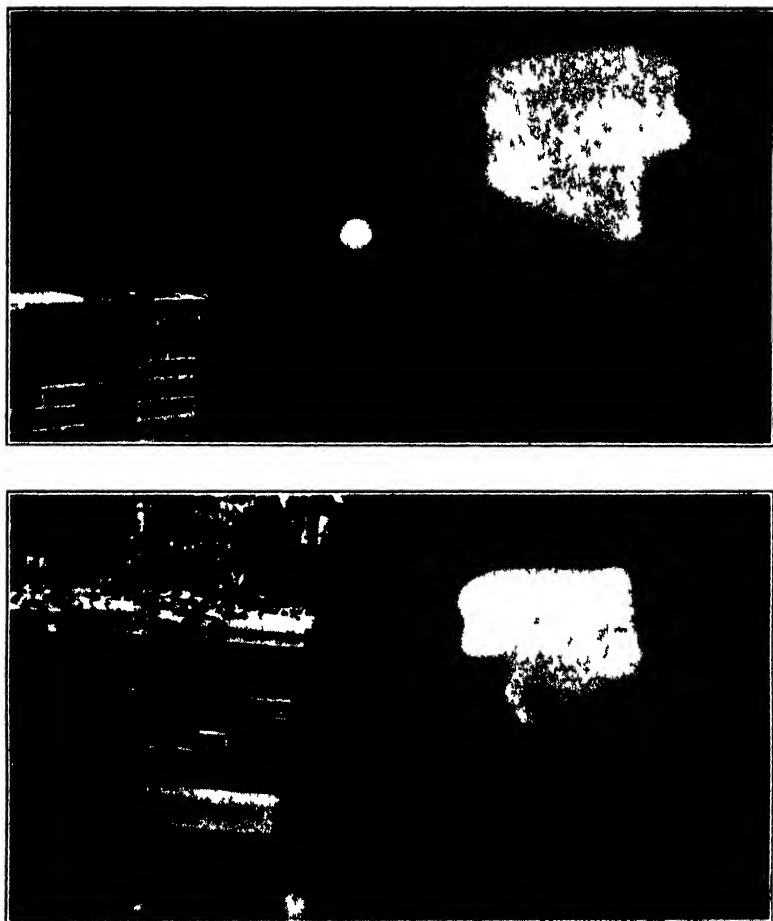


FIG 5 Photograph of right (above) and left (below) hands of R F W
(ERO files, A 2143-11, 12)

case there was a short metacarpal on both the right and left hand. Also the hand of the wife of one of the staff members of this department has a strikingly short fifth metacarpal.

Day (1915) has described and figured short fourth metacarpal bones in the right and left hands of a patient

in whom the fourth metatarsal bone was also short. A very similar defect has been described by Boorstein (1916) for the fourth metacarpal; and in another case a somewhat similar shortening of the fifth metacarpals. In Case I there was a similar defect shown in two sisters, an uncle, an aunt, grandmother of the patient and four other members of the family. In Case II, a girl of nineteen years, there was nobody in her family, so far as known, who had a similar deformity. Boorstein adds: "Her mother, in trying to explain the case of the defect, voluntarily offered the well-known explanation that it was due to some fundamental impression received by her during pregnancy (telegony¹)."

Variation in the length of the metacarpal bones has been repeatedly described; the development of radiography has called attention to scores of such cases.

The commonest type is that shown in the Fernald family. The cases of Day (1915), Miskolczy (1929^a) and Lewin (1917) show a close similarity to the Fernald case. Other cases are cited by Fontana and Vacchelli (1902), Hochheim (1904), Kenyeres (1905), Machol (1907), Mathew (1908), Chevallier (1919), Miskolczy (1929^b).

In other cases two or more metacarpals are involved in the shortening, as in Fig. 6 from Boorstein (1926). Mosenthal (1911) has described shortened 4th and 5th metacarpals, Friedländer (1916) short 3rd and 4th and 2nd and 5th metacarpals, and Miskolczy (1929^a) 3rd and 5th metacarpals.

Since Machol (1907), Chevallier (1919) and Pol (1920) have published good bibliographies and as later works are cited in Index Medicus, a complete bibliography is not needed here.

Deviations from typical development of the hand and feet are wide-spread. Brachymetacarpaly is only one type. Brachyphalangy, fusion of phalanges, curvature

¹ What is meant is "maternal impression." Telegony refers to the influence of the first sire upon the quality of subsequent offspring.

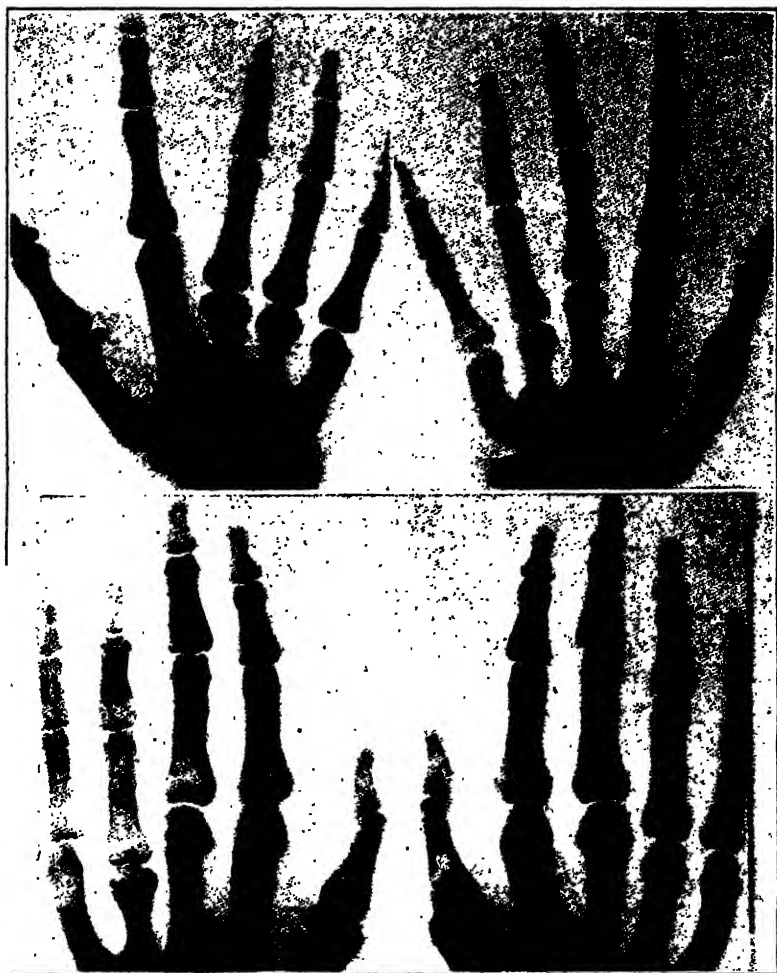


FIG. 6. *Upper*: Roentgenogram of two hands of girl 15 years of age, showing 3rd, 4th and 5th metacarpals of both hands to be short. *Lower*: Roentgenogram showing right and left hands of the father of girl in Fig. 6. The fourth fingers are short, the one on the right hand being the longer. The roentgenogram showed that the fourth metacarpal of the left hand was exactly the same shape as that of the daughter. (S. W. Boorstein, *Surgery, Gynecology and Obstetrics*, November, 1926, pp. 654-658.)

of fingers, especially of the fifth, zygodactyly, syndactyly, have been repeatedly described not only in humans but also in mice, guinea-pigs and poultry. Some of these digital defects, and others, will doubtless appear in other

animals, as more species are bred in captivity and carefully examined.

Indeed, it is remarkable that so often just five digits should appear out of the embryonic paddle, that they should be so nearly of the same relative length and should have so nearly the same bones in number and proportionate lengths. There are evidently many genes that direct the shaping of the hands and feet and if one of these is missing or modified the distal end of the appendage is modified also.

To sum up, it appears that in the Morse-Bedell branch of Mrs. Fernald's family there is a tendency toward abnormally short metacarpals; that she, as well as her daughter, inherited this tendency; that the accident which injured the fourth knuckle in Mrs. Fernald's 12th year first called attention to the short metacarpal. It is probable, however, that the short metacarpal existed before the accident. It is noteworthy, Mrs. Fernald informed me, that she noticed the short metacarpal in her daughter Elizabeth only when she had acquired the age of eight years. The hand of children is often pudgy, so that abnormalities in the growth of the metacarpals would not be readily noticed.

The case is of interest as throwing light upon the development of the idea of acquired characters. An accident calls attention, for the first time, to a defect. The defect is ascribed to the accident. Subsequently, the affected individual has a child with the same defect and this defect is ascribed to the accident that happened to the mother. The chain of evidence seems to the casual observer complete. The one fact that is missing is that the first observed defect is antecedent to the accident.

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SHORTER ARTICLES AND DISCUSSION

TWO NEW ALLELOMORPHS FOR HETEROSTYLISM IN PRIMULA

ERNST (1928) analyzed the inheritance of heterostylism in *Primula hortensis* and *P. viscosa*, of the *Auricula* group. He postulated two closely linked genes, one affecting style length, the other anther height. Brieger (1930) and Stern (1930) interpreted his results on the basis of four allelomorphic genes. Recently Ernst (1933) has published results which show the existence of six allelomorphs at this locus. The new genes differ from the old as regards their effect on the pollen grains. So far all normal thrum (short-styled, high-anthered) plants have large pollen, and all normal pin (long-styled, short-anthered) have small pollen. But each of the intermediate or homostyle types may have large or small pollen. In *viscosa* only a minority of homostyle thrum (short style, low anther) plants have large pollen, but the group of characters is inherited as a whole. Similarly, when the long-styled, high-anthered form of *hortensis* is crossed with *viscosa*, the pollen-grains associated with this type of flower are always small in later generations. The various phenotypes are given in Table I. In devising symbols for the allelomorphs the first index refers to the style, the second to the anthers, the third to the pollen. The dominant gene of normal thrums is denoted by S instead of s^{++} , and the recessive of normal pins by s instead of s^{--} . Of the eight possible genes s^{+-} and s^{-+} have not yet been described. Dominance is always complete, as regards the + characters. The various genotypes actually found in Ernst's material are given in Table I. Others are possible. For example, $s^{--}s^{++}$ should give normal thrum plants.

Two other characters were determined by this group of genes, namely, length of stigmatic papillae and fertility on crossing. The long-styled plants generally have longer papillae. One long-styled high-anthered plant had medium length papillae, but its genetics were not investigated. The relative sterility of "illegitimate" unions varies in different cases, but it is not clear whether this is due to genetical causes. Nevertheless, multiple allelomorphism as regards these characters is not impossible.

TABLE I

Species	Style	Anthers	Pollen	Genotypes
hortensis and viscosa	short	high	large	S-, s ⁺⁺ s ⁺⁺ , s ⁺⁺ s ⁺⁺
viscosa	short	low	large	s ⁺⁺ s ⁺⁺ , s ⁺⁺ s
viscosa	short	low	small	s ⁺⁺ s
viscosa	long	high	large	s ⁺⁺ s ⁺⁺ , s ⁺⁺ s
hortensis	long	high	small	s ⁺⁺ s ⁺⁺ , s ⁺⁺ s
hortensis and viscosa	long	low	small	ss

We have here another case in which the natural polymorphism of a species is due to a series of allelomorphs or very closely linked genes. Such are the allelomorphs determining self- and cross-sterility in many plants, the autosomal allelomorphs for color in *Cepea*, *Paratettix*, etc., and for blood groups in *Homo*, and the sex-linked allelomorphs for color in several fish species and the beetle *Phytodecta*. On the other hand, the natural trimorphic heterostylism of *Lythrum salicaria* and *Oxalis floribunda* is due to genes in different chromosomes, as is the tetramorphic heterostylism of the cultivated *Primula sinensis*.

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HYBRID MUTATIONS

INVESTIGATORS have often pointed out that gene mutations sometimes occur following hybridization. This seems very probable *a priori* but it is rather difficult to demonstrate experimentally. The hybrid embryo produced after interspecific crosses is,

in a sense, something more or less foreign for the maternal organism and in the F_1 hybrids the maternal contribution may be considered as being in some respects foreign for the paternal one and *vice versa*. According to the degree in which these contributions are foreign they may act very much like certain well known agents which increase the mutation rate. It is rather difficult, however, to separate the mutations induced by hybridization from the combinations also possible in the F_2 -generation.

Recently I observed a number of instances indicating hybrid mutations which seem worth reporting. These instances fall into two groups; in the first group are the plants appearing in the F_2 which are considered mutations because they carry homozygous recessive genes while the parents had homozygous dominant allelomorphs; in the second group are polymorphic trigenomal species hybrids of *Nicotiana* which originated from homozygous pure species.

First Group: Appearance of plants in the F_2 -generation carrying recessive genes in a homozygous condition while the parental plants both had dominant allelomorphs in a homozygous condition. Two instances fall into this group.

- (a) A cross of very distantly related varieties of homozygous red pepper (*dolma* and *kamby*) gave 1.37 per cent. offspring in the F_2 -generation that had orange peppers when ripe.
- (b) Two hybrids of *Secale cereale* and *Secale montanum*, homozygous for the presence of normal chlorophyll, gave a few chlorophyll albinos in the F_2 -generations. One hybrid gave 6.6 per cent. and the other gave 25.0 per cent. of these mutations, while four other sister F_1 hybrids gave only normal green progeny.

Second Group: Appearance of polymorphic trigenomal species hybrids in *Nicotiana* produced from combinations of homozygous pure species. Under this type I shall give only a few of numerous instances.

- (a) Seeds were obtained from the F_1 of *Nicotiana glauca* ($n=12$) \times *N. Langsdorffii* ($n=9$) when crossed back with *N. Langsdorffii* and 35 plants were raised. Of this number 30 were studied cytologically and all found to have 30 somatic chromosomes, i.e., 12 from *glauca*, 9 from *Langsdorffii* of the first crossing, and 9 from *Langsdorffii* of the back crossing. In *Nicotiana* the first

meiotic division is the reduction division. This first division may occasionally fail to occur in the hybrid *glauca* \times *Langsdorffii* so that at the end of the first meiotic division nuclei are formed which have 21 chromosomes, the diploid number of the hybrid. During the second division these chromosomes divide longitudinally and gametes are formed with 21 chromosomes; when such gametes are fertilized with gametes of *Langsdorffii* trigenomal plants originate. Theoretically such plants should be uniform, like the F_1 hybrids, for the component species are homozygous and each of the trigenomal plants has one genom of *glauca* and two genoms of *Langsdorffii*. However, 9 of the trigenomal plants differed morphologically from the other 21 plants which were uniform. These 9 plants differed in the shape of their leaves, the size and shape of the flowers, the length of the styles, etc. Such differences may be due to chromosome translocations, inversions, etc.; or to gene mutations. No indications were found in the somatic chromosome behavior to suggest chromosomal abnormalities. In order to separate the first types from the second the gametogenesis of these trigenomal hybrids was studied, since it is known that chromosome translocations and inversions usually lead to marked abnormalities in the reduction division. Of the 21 monomorphous trigenomal hybrids, 3 showed marked disturbances in the gametogenesis. Similar disturbances were found in 4 of the 9 polymorphic plants. The other 5 of the polymorphic plants showed gametogenesis to be similar to that observed in the other 18 monomorphous plants. These 5 plants were suspected to be hybrid mutations.

- (b) The trigenomal triple hybrids produced by crossing the hybrid *N. Tabacum* (n-24) \times *N. tomentosa* (n-12) with *N. Rusbyi* (n-12) have one genom of each of the component species, i.e., 48 somatic chromosomes. Therefore, theoretically, these plants should be uniform since the parental species were homozygous. Actually, however, two of these trigenomal plants differed in the manner already described in the preceding case.

- (c) The triple, fully fertile hybrids of *N. Tabacum* \times (*N. sylvestris* \times *N. Rusbyi*) produced recently from homozygous pure species at a temperature about 20° C. have one *Tabacum* genom (n=24), one *sylvestris* genom (n=12), and one *Rusbyi* genom (n=12), i.e., 48 somatic chromosomes. In these plants gametogenesis is regular and they should be identical, but actually the plants differed in many ways: size and shape of the leaves, color of the flowers, length of the styles and anthers, size of the whole plants, maturation period, etc.
- (d) Some of the trigenomal hybrids of (*N. Tabacum* \times *N. tomentosa*) \times *N. tomentosa* also produced from homozygous species differed markedly morphologically, although their gametogenesis was like that of the uniform trigenomal hybrids. Similar cases were also observed in other trigenomal hybrids but these and detailed data will be published elsewhere.

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OMPAX SPATULOIDES CASTELNAU, A MYTHICAL AUSTRALIAN FISH¹

O flesh, flesh, how art thou fishified!

—*Romeo and Juliet*.

SYSTEMATIC ichthyologists usually encounter sufficient difficulties in identifying natural species without having fraudulently "manufactured" fishes brought before their notice by practical jokers. In the past, several zoologists have soberly considered as actual fishes specimens, or, more often, accounts or illustrations of such, which have later proved to be mere fakes, some of which have even been given scientific names, though these, of course, have no status in modern nomenclature.

A classic instance is that of the erratic genius, Rafinesque, who was at one time the guest of Audubon at Hendersonville, Kentucky. Rafinesque tried to catch bats by beating them down with Audubon's violin, quite wrecking the instrument in his

¹ By permission of the Trustees of the Australian Museum.

enthusiasm to procure specimens. For revenge, Audubon prepared paintings of some entirely imagined fishes, which Rafinesque described as new genera and species in his "Ichthyologia Ohiensis," 1820.

Again, the generic names *Uriphaeton* Swainson 1839 (*Urophaeton* Agassiz 1846) and *Phaetonichthys* Bleeker 1876 are based on *Serranus phaeton* Cuvier and Valenciennes (Hist. Nat. Poiss, ii, Oct. 1828, p. 309, pl. xxxiv), a "species" described from a fallacious specimen in which the head of a grouper (*Cephalopholis*) has had the tail of a "flute-mouth" (*Fistularia*) attached to it.

Mermaids, Jenny Hanivers, three-eyed haddocks and faked photographs of giant fishes, trout leaping waterfalls, etc., are other traps for the unwary. These are, of course, distinct from teratological specimens, the fabulous fishes of mythology, heraldry and ancient religions, some sea-serpents, and the abstract creations of mendacious anglers, concerning all of which there is a considerable literature.

Many years ago, in the Proceedings of the Linnean Society of New South Wales (iii, 1879, p. 164, pl. xixa), Count F. de Castelnau wrote a short paper "On a New Ganoïd Fish from Queensland," as follows:

I have received from Mr. Staiger of the Brisbane Museum a drawing of a very remarkable fish, with the following note: "It is only found in a single water hole in the Burnett River, living together with *Ceratodus*; and when, in August 1872, I was in Gayndah, I got it on the breakfast table, brought in by blacks from a distance of about eight to ten miles. I had the fish for breakfast, remarked its curious shape, and asked the then Road Inspector to draw it for me, which he did. *Ceratodus*, not well known then, formed the dinner. I was not connected with any scientific body, otherwise I would have, at any rate, preserved the head. The person who drew it is not an ichthyologist but still is a draughtsman."

On examining the rough and incomplete sketch, I saw immediately that the fish was a *ganoïd* nearly allied to *Atractosteus*, but forming, by its dorsal, caudal and anal fins, all united, the type of a new genus, and probably of a new family.

It is remarkable that all the species of *ganoïd* fishes known, having a long, more or less, erccodile back, are until now, only from America. It is evident that from such a drawing no correct description can be given; all I can say is that it shows the existence in Australia of a *ganoïd* fish with a very elongate and very depressed spatuli-form snout; this is much narrower at its base than towards the two-thirds of its length; it is rounded and bordered at its extremity, having very much

the form of the beak of the *Platyus*, the two jaws are of about equal length; the eye very small and placed near the upper part of the head; the body is covered with large ganoid scales; the pectorals appear small, and are placed immediately behind and below the head; the vertical fins are very long and united, but notwithstanding, the caudal seems rather distinct; nothing is said of the dentition. Mr. Staiger says also that the fish is of a dirty mahogany color; and he adds that "*the first of the four rays is very strong*"; but I cannot find out to what this applies. The specimen was about eighteen inches long. As I have already said, the fish that comes the nearest to it is the *Atractosteus spatula* of Lacepede; much better figured by Aug. Dumins [=Duméril—G. P. W.] in his *Histoire Naturelle des Poissons*, vol. II, p. 361, pl. 24, fig. 7.

In our present knowledge of this singular fish, some inconvenience might arise from giving it a significant name; and I think it is preferable to design it under the mysterious historical one of *Ompax*. The species will bear the name of *spatuloides*.

It is much to be desired that some specimens will soon be found and secured for one of the Australian Museums.

Ompax, by its extraordinary snout, comes also near *Polyodon*, of which one species is found in the Mississippi, and another in the great Chinese river, the Yantsekiang; but these have their body naked, and cannot properly be placed with the *ganoids*.

It is singular, but almost certain, that the teeth of *Polyodon* fall before the fish acquires its full size.

Castelnau's paper naturally came in for a certain amount of criticism. The Zoological Recorder, O'Shaughnessy (*Zool. Recorder*, xvi, 1879 (1881), Pisces p. 5) opened with a broadside:

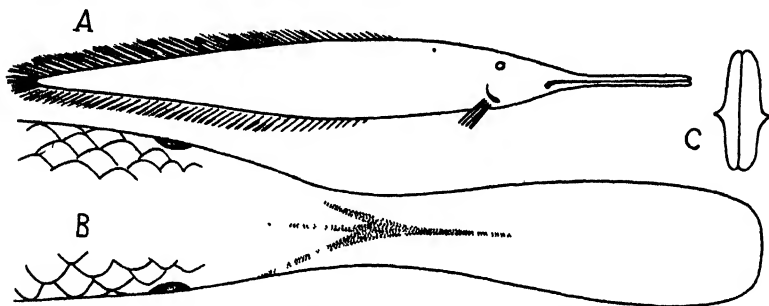
... Castelnau founds a new genus, and probably a new family, on a specimen of Ganoid fish allied to *Atractosteus* which had been eaten for breakfast by a Mr. Staiger, and all the characters of which are gathered from a drawing made after and not before the repast. He designates it "in the present state of our knowledge of this fish, by the mysterious historical name of *Ompax*." In the present state of our knowledge, the Recorder thinks he would be scarcely justified in admitting *Ompax spatuloides*, sp. n., into the system.

Sir William Macleay (Proc. Linn. Soc. N. S. Wales vi, 1881, p. 348, and Descr. Cat. Austr. Fish. ii, 1881, p. 284) remarked upon the species as follows:

1069. OMPAX SPATULOIDES, Castelnau.

The description of this genus and species will be found, with all the details known or remembered, in the Proceedings of the Linnean Society of New South Wales, Vol. III., p. 164, pl. 19 A., figs. 1-2-3. I think it is probable that no such Fish was ever found, but I give it a place in my Catalogue, in order to direct attention to the subject, as the likelihood [sic] of finding Ganoid Fishes in Queensland waters is asserted by several very distinguished Naturalists.

Ompax spatuloides has perforce been retained in lists of Australian or Queensland fishes, and figures in the most modern Check-List (McCulloch, Austr. Mus. Mem. v, 1929, p. 33), and the generic name, which Rye (Zool. Record xvi, 1879 (1881), Index new gen. p. 8) had regarded as preoccupied by *Omphax* Guénée 1857, Insecta, was included in Jordan's "Genera of Fishes" and classified with the Ceratodontidae (Genera of Fishes iii, 1919, p. 399, and Classif. Fish. 1923, p. 110).



A. Castelnau's *Ompax spatuloides* shown in lateral view.

B. Head and snout of the reputed fish seen from above.

C. Cross-section through the snout of *Ompax*.

Some months ago, my colleague, Mr. J. R. Kinghorn, of the Australian Museum, drew my attention to an article in the well-known Sydney paper, *The Bulletin* (Aug. 6, 1930, p. 21), in which the mystery of the identity of *Ompax* is solved by an author using the pseudonym "Waranbini." His account is as follows:

A sweet-scented gum (*E. staigeriana*) of the northern parts of Queensland perpetuates the memory of Carl Theodore Staiger, who was at one time director of Brisbane Museum, and was made the victim of one of the quaintest jokes in scientific history. While he was visiting Gayndah station the hard-cases there prepared a new fish for Staiger, made of the head of a lung fish, the body of a mullet and the tail of an eel. It was nicely cooked and placed before him for his breakfast, with the remark that it was something new—a very rare fish that had never been seen anywhere but at Gayndah. Mr. Staiger was immensely interested, and expressed regret that he had not seen it alive. On being told that it might be months before another was caught, he made a careful sketch of the cooked specimen before he started to eat it. The sketch and a description were subsequently sent to an ichthyological expert, Count Castelnau, and that gentleman named it *Ompax spatuloides*. Whenever a marine [!] mystery was captured afterwards in the Gayndah district, the locals would remark with a grin that it "must be an Ompax!"

From this account, it is obvious that *Ompax spatuloides* is a purely mythical genus and species, whose disgraced name must be removed from the Australian list.

The account given by "Waranbini" contains one or two minor discrepancies when compared with Castelnau's but these may be due to a lapse of memory which is pardonable when it is recalled that half a century had passed between the times of appearance of the two. In the modern account, for instance, the head of a lung fish is said to have been used in the preparation of *Ompax*, but to the present writer it seems more likely that the head of some fresh-water Long Tom (family *Belonidae*), or perhaps even a *Platypus* bill, may have been utilized in this strange concoction.

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THE EFFECTS OF ALPHA-RADIATION FROM POLONIUM ON THE DEVELOPING EGGS OF *DROSOPHILA MELANOGASTER*¹

WHILE the biological effects of radiation from other members of the radium family have been studied widely in recent years, alpha-radiation from polonium is still the object of relatively rare investigation.

Lacassagne and his associates² (Fondation Curie, Institut du Radium, Paris), studying the effects of polonium solutions injected into the organism, have observed (1924-1928) profound changes in the blood and histological changes in certain organs, particularly glandular tissues and organs of excretion: the kid-

¹ The expenses of this investigation were supported in part by a grant from the committee on the effects of radiation upon living organisms of the National Research Council.

² A. Lacassagne and J. Lavedan, "Les Modifications Histologiques du Sang Consécutives aux Irradiations Experimentales." *Paris Médical*, 51: p. 97, 1924; *ibid.*, "Modifications Numériques des Elements du Sang Sous l'Influence des Irradiations Experimentales." *La Médecine*, No. 9, June, 1924; A. Lacassagne, J. Lattés and J. Lavedan, "Etude Experimentale des Effets Biologiques du Polonium Introduit dans l'Organisme." *Jour. de Rad. et d'Elect.*, 9: 1-82, 1925; A. Lacassagne, "Modifications du Sang Déterminées par les Rayons-X et les Corps Radio-actifs." *Progrès Médicale*, 30: 1077-1087, 1928.

ney, the skin, the thymus, the genital and adrenal glands, the liver and also the lungs. Emile-Weil and Lacassagne³ (1925) recorded the production of grave anaemia and leucemia due to numerical modifications of the blood-elements and because of injuries to the blood-forming tissues.

In investigation of the action of radium salts on colloids, Hardy⁴ incidentally discovered the effects of alpha-radiation alone upon globulin solutions, rendered acid or alkaline by the addition of dilute acetic acid or ammonia. When the solutions exposed to 50 mgms. of radium bromide were separated from the radiation source by two thin layers of mica of the containing capsule (which would shut out all alpha-rays), no changes were perceptible even after one hour's exposure. When, however, samples of the globulin solutions were exposed as naked hanging drops, changes were apparent at the end of three minutes' time. The acid solution became clearer, showing thereby more complete solution. The alkaline solution turned to an opaque jelly. The physico-chemical explanation of this phenomenon is interesting in itself, but from the biological point of view such a result may be considered important as showing that exposure to alpha-radiation produces effects which differ both in kind and in degree from those caused by exposure to the beta and gamma-rays.

Lacassagne and Desclin⁵ (1928) exposed the eggs and young embryos of frogs to alpha-radiation from polonium. They obtained destruction of localized areas with minimum traumatism. While entodermal invagination was perfectly normal, as development proceeded, these localized areas produced malformations of specific organs or regions, especially the olfactory organ, the optic and auditory vesicles, the cervical and branchial regions.

Swann and del Rosario⁶ (1931), in studies upon the effects of radioactive substances upon *Euglena*, have found that alpha-particles kill the animals in proportion to the strength of the

³ P. Emile-Weil and A. Lacassagne, "Anémie Pernicieuse et Leucémie Myéloïde Mortelles Provoquées par la Manipulation de Substances Radioactives," *Bull. de l'Acad. de Méd.*, 93: No. 9, 1925.

⁴ W. B. Hardy, "The Action of Salts of Radium upon Globulins," *Proc. Physiol. Soc.*, May 6, 1903: 29-30.

⁵ A. Lacassagne and L. Desclin, "Sur l'Emploi des Rayons-Alpha du Polonium en Embryologie, comme Agents de Destructions Localisées," *Compt. Rend. Soc. de Biol.*, 99: p. 98, 1928.

⁶ W. F. G. Swann and C. del Rosario, "The Effect of Radioactive Radiations upon *Euglena*," *Jour. Franklin Inst.*, 211: 303-317, 1931.

dissolved emanation present in the water. Rona (Institut für Radiumforschung, Vienna) finds that the spores of the mushroom, *Mucor*, are killed by five minutes' exposure to polonium (unpublished results). Other research workers associated with her (Blau, 1931⁷; Blau and Kara-Michailova, 1931⁸) have studied physical aspects of the radiation from polonium.

The present authors are investigating the effects of alpha-radiation from polonium upon *Drosophila* eggs (wild-type flies) in different stages of their development. During preliminary experiments to determine the dosage in exposure time the spores of two species of mould were irradiated. Fifteen minutes' exposure killed the spores so that no cultures developed. Cultures already growing were prevented from fruiting and soon died out.

The polonium used was radium F, a substance which gives off practically only alpha-rays. It is the last radioactive element of the long radium family, its products of disintegration being helium and lead. The mean life of solutions of this polonium is 196 days. From a suitable solution the polonium is deposited on a silver disk. This in turn is attached to a silver plunger, and the whole is enclosed in a small brass cylinder, which is mounted with adjustable screws. Eggs and spores were treated in petri dishes on an agar medium. The adjustable brass cylinder and the plunger arrangement enable the disk to be brought into close proximity with the surface of the culture medium. Radiation effects upon eggs in two stages of development have been studied especially: (a) collected within a quarter of an hour after laying from a large number of females of the same age—eggs fertilized, divisions just beginning—*pre-blastula stage*; (b) collected two and one half hours after laying—blastula complete, polar cells aggregated—*blastula stage*. These stages were identified in time from cytological studies made previously in connection with other experiments, which accord almost exactly with those recorded by Geigy (1931⁹) in

⁷ Marietta Blau, "Über das Abklingen des latenten Bildes bei Exposition mit Alpha-Partikeln." *Sitzungs. der Akad. der Wissenschaft.* Wien, 140: 623-628, 1931.

⁸ Marietta Blau and E. Kara-Michailova, "Über die durchdringende Strahlen des Poloniums." *Sitzungs. der Akad. der Wissenschaft.* Wien, 140: 615-622, 1931.

⁹ Rodolphe Geigy, "Action de l'Ultra-violet sur le Pôle Germinal dans l'Oeuf de *Drosophila Melanogaster*." Genève, Albert Kundig, 1931.

his investigations of the action of ultra-violet light on the germinal pole of *Drosophila* eggs. The individual differences in eggs collected in this way are not great.

Eggs were exposed in the pre-blastula and the blastula stages to irradiation of five and fifteen minutes' duration. The experimental results given briefly in the following table indicate a higher sensitivity in the blastula stage as shown by the greater percentage of eggs failing to hatch. In each instance where irradiation was made in the blastula stage, all the larvae hatching from irradiated eggs died within three hours after hatching. Where irradiation was made of eggs in the pre-blastula stage, all the larvae hatching survived to pupate and become adult flies.

No. eggs	Stage	Exposure time	Electro-static units	Per cent. of eggs hatching	Remarks
100	pre-bl.	5 min.	2,604	56	living flies
100	blast.	5 "	2,604	30	no living flies
100	pre-bl.	15 "	2,604	38	living flies
100	blast.	15 "	2,604	15	no living flies
100	pre-bl.	7.5 "	1,543	53	living flies
100	blast.	7.5 "	1,543	27	no living flies
100	pre-bl.	22.5 "	1,543	29	living flies
100	blast.	22.5 "	1,543	20	no living flies

Of these, however, some in each case showed somatic deformities: 24 out of the total 176 living flies, or a percentage of 13.6363 ± 1.74466 . The malformations produced consisted of twisted head or thorax, varying degrees of the absence of wings with the remnants crumpled and torn, and in one case absence of legs on one side of the body. This one individual, a male, was non-viable and died two days after emergence. All others gave normal viable offspring, there being no indication in three generations that the defects were heritable. No sterility in the adult fly was brought about by exposure to alpha-radiation. The deformities produced recall those reported by Lacassagne and Desclín (1928) in studies of the effects of alpha-radiation upon the eggs and young embryos of frogs.

Thus the results of exposure to the alpha-rays of radium F differ considerably from those brought about by beta or gamma radiation. This is a brief preliminary report; the experiments are being continued and extended.

The authors find pleasure in expressing their appreciation to members of the staff of the Curie Foundation Institute for the opportunity to carry on the investigations reported here.

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THE INHERITANCE OF DICHROMATISM IN THE DEER-MOUSE, *PEROMYSCUS MANICULATUS BLANDUS*¹

THE occurrence of two or more color phases in one species at the same geographical locality has been described for a number of kinds of mammals. A well-known example is the gray squirrel of eastern North America, which in many localities has both gray and black phases.

In the mice of the genus *Peromyscus*, Osgood² has described the occurrence of two color phases in *Peromyscus maniculatus gambelii*, in *P. m. blandus*, in *P. m. sonoriensis* and in *P. m. coolidgei*. In none of these races are the two color phases sharply alternative, for in each race some specimens are intermediate in color tone.

The buff and the gray color phases of *Peromyscus m. gambelii* from southwestern California were each found by Collins³ to breed true, but when these extremes were hybridized, the offspring were intermediate in color, and the mice of the F₂ generation likewise were intermediate between the two color phases.

Peromyscus maniculatus blandus is said by Osgood to have a buff color phase, which is similar to the color of *sonoriensis*, and a gray phase, which is unique. "All stages of variation between the two phases occur." Both of these color phases occur at Alamogordo, New Mexico, where 56 mice of this subspecies were collected by the University of Michigan-Walker-Harris Expedition of 1927.⁴ Some of these animals were brought to Ann Arbor alive and became the parents of an extensive stock; others were put up in the field as round or flat skins. Of the total

¹ The completion of this study was made possible by a research grant from the American Association for the Advancement of Science.

² W. H. Osgood, *U. S. Dept. Agric., North Amer. Fauna*, No. 28, 1909.

³ H. H. Collins, *Jour. Exp. Zool.*, 38: 91-92, 1923.

⁴ L. R. Dice, *Univ. Mich., Occ. Papers Mus. Zool.*, No. 213, p. 25, 1930.

number of field-caught mice prepared as specimens, 9 are too immature for certain determination of their color phase; 16 are certainly buff, 20 are gray, and 11 are more or less intermediate between buff and gray. Osgood's statement that all intermediate stages of color occur is true, for no sharp line between buff and gray can be drawn in this series.

Among the breeding stock of *blandus* brought to the University of Michigan, two females were buff, while all the other animals were gray. By mating these buff females to gray males some buff offspring were secured. Matings of gray with gray produced gray offspring, and therefore the possibility was suggested that gray was recessive to buff. Extensive further matings have tended to confirm this hypothesis.

From matings of gray \times gray I have records of 41 gray females and 42 gray males, and also 1 buff male. This buff individual was not saved as a specimen, and possibly was wrongly identified. From matings of buff \times buff I have records of 3 buff females and 2 buff males. Evidently the sex ratios are about equal in both the gray and the buff phases.

From matings of gray \times buff (heterozygous for gray) were obtained 73 gray, 76 buff, and two intermediate (expected 75.5 gray:75.5 buff). From gray \times buff (possibly heterozygous for gray) were secured 30 gray and 31 buff (expected 30.5:30.5). From matings of gray \times buff (probably homozygous) were obtained 14 buff (expected 14 buff). These results indicate that gray is recessive to buff and that the two colors are allelomorphs, behaving in inheritance as a simple mendelian character difference. There is, however, a discrepancy due to the occurrence of a few intermediates. These perhaps may be due to modifying factors, which mask the clear expression of the color phases.

Intermediates are not so numerous among the laboratory-bred mice as in those collected in the field. This may partly be owing to the fact that the laboratory-bred animals are of known age and therefore are certainly fully mature, while some of the wild-killed animals are obviously immature. I have found that color phase determinations are often erroneous if made before the mice are six months of age.

Another possible reason for fewer intermediates in the laboratory-bred stock is that the laboratory parents were selected both for buff and for gray, and those of intermediate character were

not used for breeding. If, as is probable, the *blandus* stock contained various color modifiers, these would tend to be eliminated by the selection, leaving buff and gray to show in a less obscured manner.

A color modifier of proved potency has actually been found in the stock. This is a dilution factor affecting both the gray and the buff phases. Its principal effect is greatly to reduce the amount of black on the tips of the hairs, so that the animals appear much paler. With the amount of black in the pelage reduced, the difference between the gray and buff color phases is much more apparent.

The dilute character was first noted in several offspring from a female, the daughter of two field-caught mice, mated with her son, who was fathered by her brother. There was therefore much inbreeding in this strain, and it is probable that the dilution factor was present in the original wild stock.

The dilution factor seems definitely inherited, and while its genetics have not been worked out, it seems to behave as a single-unit Mendelian recessive to full color. From matings of dilute \times dilute 18 offspring are dilute, as would be expected under this hypothesis.

Another color modification which has arisen in the *blandus* stock is a white spot on the crown of the head. Similar white spots on the head have been found in many different subspecies of *Peromyscus maniculatus* and *P. leucopus*, sometimes in specimens taken in the field. However, I have not found such white head spots in wild *blandus*. The white spot arising in the laboratory-bred *blandus* is definitely inherited. From parents both of which had white spots on the crown 8 young all had varying amounts of white on this part of the head.

The white spots occurring on the head in other races of *Peromyscus* are also inherited, though in a variable manner. Sometimes the occurrence of a white tip to the tail seems to be correlated with the occurrence of the white spot on the head. By selection it seems possible to increase the size of these white spots to some extent. Sumner⁵ also has noted the occurrence of white spots on the head in "various races of *Peromyscus*," and Castle⁶ states that "The production of white-spotted races from small

⁵ F. B. Sumner, *Bibliographia Genetica*, 9: 38, 1932.

⁶ W. E. Castle, "Genetics and Eugenics," ed. 2, p. 125, 1920.

beginnings observed in wild stocks has been accomplished in the laboratory by Castle and Phillips in the case of *Peromyscus*.''

While the occurrence of the character for white spotting in wild deer-mice probably does not ordinarily affect the color of much of the animal, yet it is very likely that numerous other hereditary color variations occur, and some of these, like the dilution factor, probably do affect the expression of the color phases. It seems justifiable, therefore, to assume that some unknown color modifiers cause the production of the apparent intermediates between the color phases.

A great deal of variability in hereditary factors undoubtedly occurs in all wild populations of *Peromyscus*. In another paper⁷ I have demonstrated that in a population of *Peromyscus m. rufinus* taken in Colorado, there is a large amount of genetic variability in size factors and some variability in color factors. The dichromatism of *blandus* is another illustration of the extent of genetic variability in the wild populations of these deer-mice.

SUMMARY

The extremes of the gray and buff color phases of the deer-mouse, *Peromyscus maniculatus blandus*, behave in heredity as Mendelian allelomorphs, gray being recessive to buff. However, among the experimental animals several can not be assigned with certainty to either color phase, and in the series of specimens of wild animals there is an appearance of complete intergradation between the two color phases.

Several inherited factors modifying color and pattern are shown to occur in the subspecies, and it is assumed that other unknown modifying factors mask in some individuals the clear expression of the color phases, and thus produce the apparent intermediates.

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⁷ L. R. Dice, *Univ. Mich., Occ. Papers Mus. Zool.*, No. 271, in press.

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